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Hippocampal coding of positions of inaccessible objects

Hipokampální kódování pozic nepřístupných objektů

Diploma thesis

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PROHLÁŠENÍ

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Podpis

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ABSTRACT

The survival of the animals depends on their ability to memorize locations and to use behavioral spatial navigation strategies. The crucial structure for this type of behavior and memory is the hippocampus, by its ability to create a cognitive map. In this structure, there are specialized pyramidal cell called place cells. They respond by their complex firing pattern to specific animal's location in the environment. Even though many studies have investigated the role of hippocampal pyramidal cells in spatial navigation and object position discrimination, their function during inaccessible object position discrimination is not yet clarified. In our experiment, rats were trained in a behavioral task to discriminate between rewarded and nor-rewarded positions of object located in an inaccessible space. We investigated the role of individual hippocampal cells during this task by single neuron electrophysiology.

The first aim of this study was to decide which of two configurations of objects presented on a computer screen during spatial object discrimination task rats can discriminate easily. The second aim is to show whether and how information about the position of inaccessible objects is represented in the hippocampus using single-neuron electrophysiology.

We found out, that animals did not reach learning criteria in the one-dimensional object-position discrimination (four objects equally spaced from left to right side on a computer screen). On the contrary, the rats fulfilled the learning criteria in the two-dimensional object-position discrimination (four objects displayed in the corners of the computer screen). This observation can be explained by the different distance between discriminated positions and their configuration. During two-dimensional object position discrimination, single neuron analysis shown that pyramidal cells in CA1 specifically responded by their firing activity to rewarded, or non-rewarded positions, but we have not found any single stimulus related activity. This could be explained by categorization of rewarded and non-rewarded positions. These results suggest that CA1 pyramidal cells play a crucial role in the discrimination of positions of objects located in inaccessible space.

Keywords: hippocampus, spatial cognition, object-position discrimination, inaccessible space, neuronal ensembles, electrophysiology

ABSTRAKT

Schopnost zapamatovat si konkrétní prostředí a využít behaviorálních strategií k prostorové navigaci jsou nezbytné pro přežití ve volné přírodě. Za pomocí tvorby kognitivních map tyto procesy zprostředkovává hipokampus, jehož funkce je úzce spojována s chováním a pamětí. V hipokampu se nachází specializované pyramidové buňky, kterým se říká neurony místa. Tyto buňky reagují na pozici jejich vlastníka v prostoru jejich charakteristickou komplexní salvou akčních potenciálů. I přestože existuje mnoho studií zabývajících se jejich nezbytnou rolí v prostorové navigaci a v rozeznávání pozic objektů, jejich funkce během rozeznávání pozic objektů v nepřístupném prostoru nebyla dosud objasněna. Během našeho experimentu, potkani byli v behaviorální úloze trénováni rozeznávat odměňované a neodměňované pozice umístěné v nepřístupném prostoru. V této práci jsme zkoumali roli jednotlivých hipokampálních pyramidových buněk, během této úlohy, za pomocí měření jednotkové aktivity.

Prvním cílem této studie bylo rozhodnout, který ze dvou typů konfigurací, prezentovaných během úlohy rozeznávání pozic objektů na počítačové obrazovce, potkani dokážou lépe rozlišit. Druhým cílem bylo ukázat, jak je informace o pozici objektu v nepřístupném prostoru reprezentována v hipokampu za využití elektrofyziologické metody měření jednotkové aktivity.

Zjistili jsme, že potkani nedokázali dosáhnout námi stanovených kritérií během učení v jednorozměrném rozpoznávání pozic objektu (jednalo se o čtyři rovnoměrně rozmístěné obdélníky z pravé do levé části obrazovky v rámci jedné osy). Naopak potkani s dvoudimenzionálním tréninkem (čtyři kruhy v rozích obrazovky) dokázali tato kritéria s vysokou úspěšností dosáhnout. Toto zjištění může být vysvětleno jinou vzdáleností mezi rozlišovanými pozicemi objektů a jejich konfigurací. Během rozpoznávání objektů ve dvojrozměrné konfiguraci analýza jednotkové aktivity ukázala, že pyramidové buňky v CA1 oblasti hippokampu specificky odpovídají na odměňovanou nebo neodměňovanou pozici, avšak aktivita spřažená pouze s jedním typem stimulu nebyla nalezena. Toto pozorování může být vysvětleno kategorizací odměňovaných a neodměňovaných pozic. Tyto výsledky naznačují, že pyramidové buňky z CA1 oblasti hrají zásadní roli v diskriminaci pozic objektů v nepřístupném prostoru.

Klíčová slova: hipokampus, prostorová kognice, rozlišování pozice objektu, nepřístupný prostor, populace neuronů, elektrofyziologie

LIST OF ABBREVIATIONS

CA	Cornu ammonis
CH	chattering neurons
DG	dentate gyrus
FS	fast spiking neuron
GABA	γ-aminobutyric acid
HD	head direction cell
LED	light emitting diode
LFP	local field potential
IB	intrinsically bursting neuron
Non-Rew	non rewarded position
SEM	standard error of mean
RS	regular spiking neuron
Rew	rewarded position
SPW	single sharp wave event

CONTENT

THEORETICAL PART

1. INTRODUCTION 2
2. SPATIAL NAVIGATION 2
3. HIPPOCAMPUS. 4
   1.1 Hippocampus - part of evolutionary conservative system 4
   1.2 Hippocampal morphology and neuronal types 6
      1.2.1 Trisynaptic circuit 6
   1.3 The main neuronal types in the hippocampus 8
      1.3.1 Pyramidal cells. 8
      1.3.2 Basket cells 9
   1.4 Function of the hippocampus 9
   1.5 Memory 9
   1.6 Spatial navigation is also memory 10
2 Hippocampal role in spatial navigation tasks 11
   2.1 Navigational tasks 11
      2.1.1 T-maze 11
      2.1.2 Radial maze 12
      2.1.3 Morris water maze 12
      2.1.4 Carousel maze 13
      2.1.5 Object- position recognition tasks 13
      2.1.6 Object position recognition using touch-screen presentation 14
3 THE NEURAL BASIS OF SPATIAL REPRESENTATION IN RODENTS 16
   3.1 Place cells 16
   3.2 Grid cells 17
   3.3 Head direction cells 18
   3.4 Boundary cells 18
   3.5 Object vector cells 19
4 ELECTROPHYSIOLOGY 19
   4.1 Electrophysiology as a tool to understanding brain 19
   4.2 Electrophysiological properties of neurons 20
   4.3 Hippocampal oscillatory activity 20
      4.3.1 Theta oscillations 20

4.3.2 Gamma oscillation 21
4.3.3 Sharp waves and ripples 21
4.4 Hippocampal CA1 as a crucial region in spatial navigation 22
4.4.1 Pyramidal cells 22
4.4.2 Theta cells 22
5 AIMS OF WORK 24
6 Materials and methods 25
6.1 Subjects 25
6.2 Behavioral apparatus 25
6.3 Experimental design 26
6.4 Surgery 27
6.5 Implants 28
6.6 Recording techniques. 29
6.7 Data analysis 30
7 Results 31
7.1 Learning phase 31
7.2 Recording phase 32
7.3 Electrophysiological validation of tetrode location 40
8 Discussion 41
9 Conclusions 43
10 REFERENCES 44
10.1 Figures 57

THEORETICAL PART

1. INTRODUCTION

The role of the hippocampus in spatial navigation has been investigated for many decades. The hippocampus plays a crucial role in multiple neural processes such as episodic memory, cognition, behavior, and spatial navigation (Rubin et al., 2014). During extensive studies of this important structure, the existence of neurons that fires in specific places were discovered. These neurons are so-called place cells. Together with other spatial navigation related neurons (e.g. grid cells, head direction cells, boundary cells) they form a cognitive map of the environment (Tolman, 1948) (Park et al., 2011). In the hippocampus proper, they can be found in the CA1 and CA3 regions. Morphologically they are pyramidal cells with characteristic complex spike bursts. Previous experiments confirmed importance of hippocampal pyramidal cells in spatial navigation tasks. Animals perceive positions objects not only at the places they can visit but also in inaccessible spaces. It is still not fully clarified how the hippocampal pyramidal cells are involved in discrimination and remembrance of those distant objects' positions. For this purpose, we used an inaccessible object position discrimination task. The goal of this thesis is to elucidate whether and how exactly are CA1 pyramidal cells involved in the discrimination of positions of objects located in inaccessible space with the help of an electrophysiological technique called single-unit recording.

2. SPATIAL NAVIGATION

Virtually all animals need to map their environment and space. Any moving animal must be able to plan its path, which leads it to the nest, water sources, a predictable position of prey, predators, and meting partners, etc. The capability of spatial orientation implies that animals must have some mechanisms of long-term knowledge of space (memory), responsible for navigation to and away from particular places.

For long-distance (geographic) navigation, some animals possess special organs that help them detect changes in the magnetic field, gravity, or ocean currents (Alerstam et al., 2003). Animals that do not have such specialized organs use marks like stars, sun, or moon, specific odors usually combined with communication among the individuals for long-distance navigation.

In the case of navigation over smaller scale distances (topographic navigation), there are two major ways how to navigate: the egocentric (idiothetic) and allocentric (allothetic) navigation.

Egocentric navigation is sometimes referred to as fixed because it is self-centered. The environment is mapped to the individual's location perspective. The location of the objects is defined by an angle that is measured concerning the individual's position (Mittelstaedt & Mittelstaedt, 1982). This way of spatial

cognition is essential for memorizing the routes based on sequential turns, that is why it is also called route-based strategy.

Allocentric navigation, or map-based strategy, use external landmarks and cues and their mutual relations and how they related to the animal, and, in the contrast to egocentric navigation, it is not dependent on the current position of the subject. The advantage of this navigation is flexibility, (such as the ability to locate novel points from different start locations) and high informational value. The disadvantage is that it requires a relatively stable environment (Klatzky, 1998) (Iglói et al., 2009).

In connection with spatial navigation, it is important to mention the path integration strategy. It is the capacity to use idiothetic cues to update the calculation of the animal location by monitoring its trajectory concerning start location (Etienne & Jeffery, 2004). This strategy is connected to the egocentric navigation, but the combination with the allocentric navigation is free from idiothetic cumulative mistakes (Stuchlik & Bures, 2002).

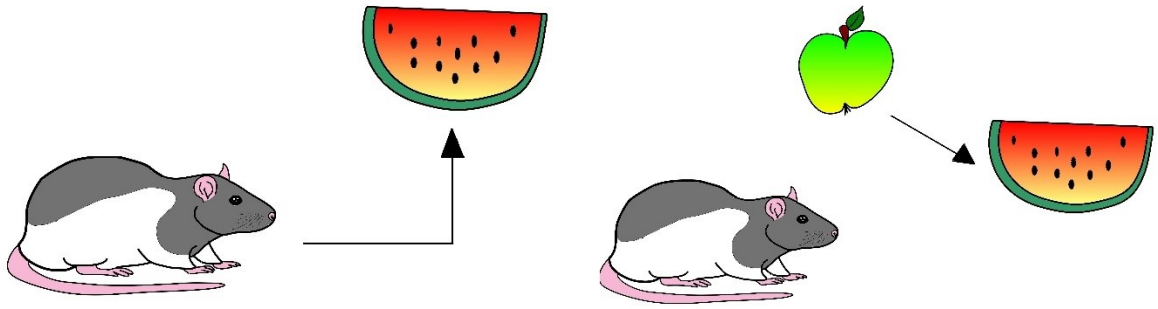


Fig 1: Difference between egocentric and allocentric navigation. *Egocentric (left picture) navigation represents the location of the object in space relative to the rat's location ("watermelon is on the left side in front of me"). Allocentric navigation (right picture) encodes object's location in respect to other objects, independently to the subject location ("the watermelon is next to the apple").*

Edward Tolman in 1948 (Tolman, 1948) hypothesized that spatial behavior is driven by internal representations, organized as a cognitive map. This idea was against the stimulus-response association theory (Spence, 1950) that was accepted by many researchers those days. A cognitive map is a mental representation of the environment in which an animal navigates. By visualizing the landscape by these maps, it is much easier for the animal to solve orientation problems and generate shortcuts and detour behavior (Tolman, 1948). Of course, not every animal is able of this and it is generally considered that

mammals (including humans) and birds have such complex navigational mechanisms (Jacobs, 2003). The main structure responsible for spatial navigation and cognitive map representation is the hippocampus. (Colombo & Broadbent, 2000). There are some exceptions, like bees, that seem to be also able to form some similar maps, but the brain structure responsible for this behavior is not the hippocampus as in the previously mentioned cases (Breed & Moore, 2012).

In the seventies, the connection between the hippocampus and spatial navigation was proposed, since the initial lesion studies showed impairments in spatial learning and memory tasks (Milner, 1965). Performance in non-spatial tasks, that do not require the hippocampus, was unaffected (O'Keefe, 1978). Later this view has been questioned (Dusek & Eichenbaum, 1997), but the prevailing view today is that the hippocampus is essential for declarative, mainly episodic (Scoville and Milner, 1953) and spatial memories (Nedelska et al., 2012) in humans, and spatial and other relational forms of memory in animals (Bunsey & Elchenbaum, 1996). The following section of the literature overview will discuss various aspects of the structure and function of the hippocampus.

3. HIPPOCAMPUS.

The hippocampus plays a central role in neuroscience research, due to its crucial role in memory, spatial navigation, cognition, and behavior. Moreover, it is conveniently organized into distinct subfields and layers, facilitating localized access for manipulating and registering neural activity. The term hippocampus is derived from the Greek word for sea horse, due to the anatomical resemblance of the human hippocampus to this marine fish. Another Latin name for the hippocampus is "Cornu ammonis" translated to Ammon´s horn due to the shape of the hippocampus. This title is also still used as a differentiation of the subfields of the hippocampus to CA1, CA2, and CA3 (Cornu ammonis). (Schultz & Engelhardt, 2014). CA subfields of the hippocampus are often summarized as "hippocampus proper", which in contrast to the simplified term "hippocampus", does not describe dentate gyrus as a part of the complex. (Walther, 2002).

1.1 Hippocampus - part of evolutionary conservative system

Hippocampus is part of allocortex (evolutionary older than neocortex), located between the cerebral cortex and thalamus. Even though the location, size, or form differ between vertebrates, the basic layout of this formation and hippocampal role in spatial navigation is evolutionary conservative and it makes hippocampus a crucial for surviving (Bingman, 1992) (Rodríguez et al., 2002) (Lefebvre et al., 2004).

The hippocampus is a major part of the complex brain binate structure called hippocampal formation. This complex consists, except for the mentioned hippocampus, of dentate gyrus (DG), presubiculum, subiculum, parasubiculum, and entorhinal cortex. But some authors description of the hippocampal formation is limited just to three layers parts - hippocampus, subiculum, and gyrus dentate. Subicular parts are six layers structures and they are summed in this view by term parahippocampal part (Witter & Amaral, 2004).

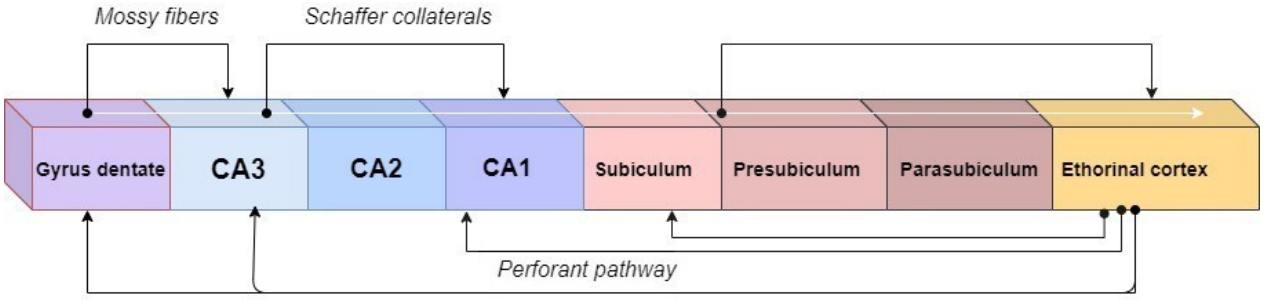


Fig2: Schematic picture of the connection circuit of the hippocampal formation. *Neurons from the entorhinal cortex project via the Perforant pathway (Neurons from II-layer project to Gyrus dentate and CA3 field and neurons from III-layer of the entorhinal cortex project to the CA1 field). Granule cells then connect gyrus dentate and CA3 by Mossy fibers connections. Then pyramidal neurons from CA3 project to CA1 via connections called Schaffer collaterals. CA1 pyramidal cells project to the Subiculum. And by connections between CA1 and subiculum and entorhinal cortex the circuit is closed. Picture by author inspired from (Amaral, 2007).*

There are multiple ways how to differentiate a hippocampus to parts. But for this thesis, the most important one is to ventral and dorsal parts. The dorsal part corresponds to the posterior part of the hippocampus in primates and the ventral part to the anterior part. Some authors add one more part – intermediate region, which partly has overlapping characteristics of the neighboring parts (such as place learning and spatial navigation) (Bast et al., 2009). Dorsal hippocampus has a main role in cognitive functions (Gilbert & Kesner, 2003), the lesion or inactivation of the dorsal hippocampus reflects on impaired spatial navigation (Rogers & Kesner, 2006), active place avoidance (Cimadevilla et al., 2000), learning (Lee et al., 2005) and episodic-like memory processing (Li & Chao, 2008). Since the dorsal hippocampus is the main structure responsible for spatial navigation it will be the region of interest in the practical part of this thesis.

The ventral hippocampus is connected to emotional processing and behavior (Fanselow & Dong, 2010). Lesion studies have shown disrupted decision-making (due to impaired motivation behavior) (McHugh et al., 2008) and decreased contextual fear conditioning (Anagnostaras et al., 2001).

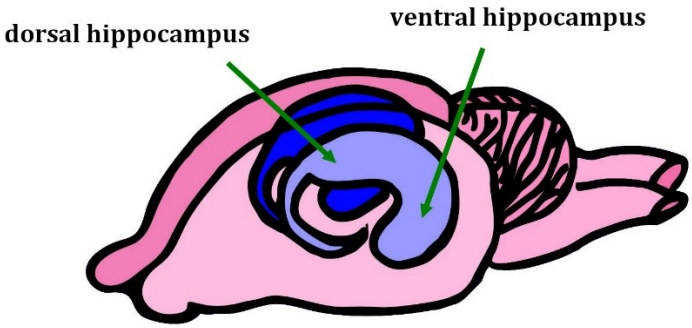


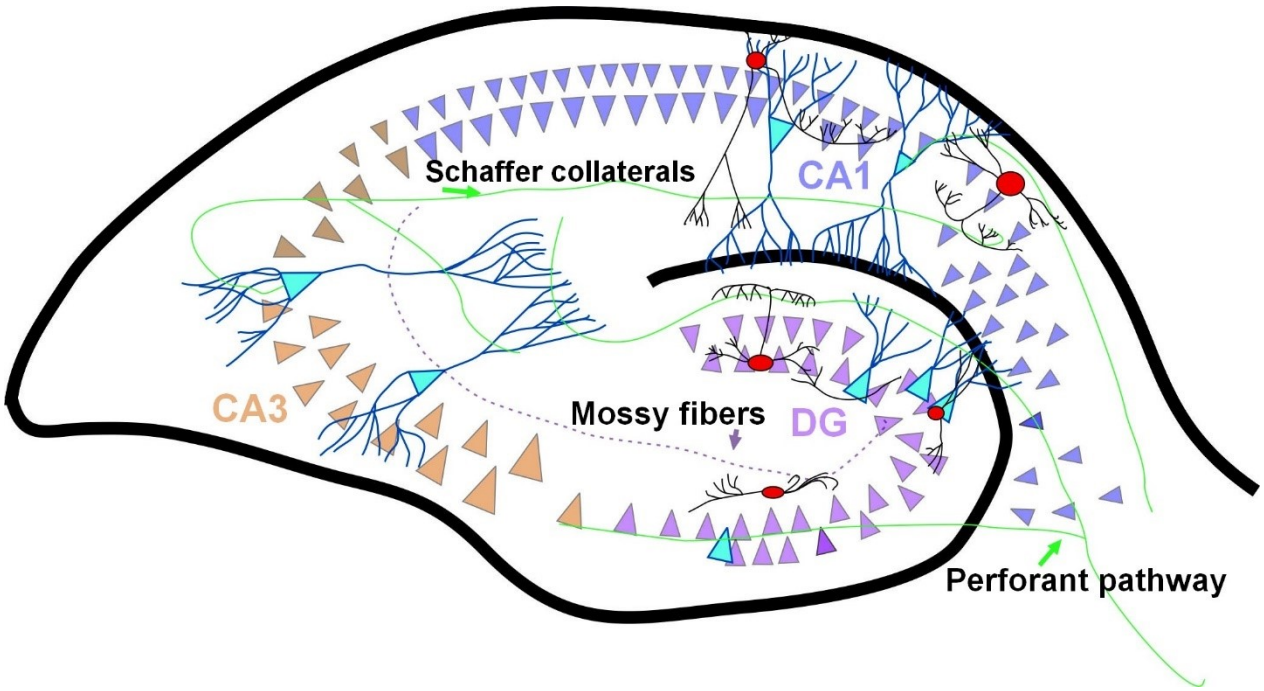
Fig 3: Schematic picture of the rat brain shows the localization and size of the hippocampus.

1.2 Hippocampal morphology and neuronal types

As mentioned before, the hippocampus is divided into three parts – CA1, CA2, and CA3. Some authors also talk about a fourth region - CA4. However, its location coincides with the deep polymorphic region of gyrus dentate. The existence of region CA2 was also controversial, but multiple studies supported the presence of this region by connectional and functional data (Bartesaghi & Gessi, 2004), and gene- expression analysis shows the differentiation of the region as a separated field (Lein et al., 2007).

1.2.1 Trisynaptic circuit

The trisynaptic circuit is a trisynaptic loop connecting the parts of the hippocampus. It consists of granule cells from gyrus dentate, pyramidal neurons from CA3, and pyramidal neurons from CA1. The dentate gyrus receives information from the entorhinal cortex via the perforant pathway (see Fig.2), where the trisynaptic loop begins. Granule cells from dentate gyrus than projects to the CA3 by Mossy fibers. It is a term for unmyelinated axons with excitatory function, projecting from granule cells that terminates in CA3 (Alle et al., 2009). Then CA3 is connected to the CA1 by Schaffer collaterals. Schaffer collaterals are not, same as mossy fibers, neurons, but projections. They are axons of CA3 pyramidal cells, that connect this region to the CA1 pyramidal cells. The supreme neurotransmitter of these connections is glutamate. (Onodera et al., 1986). Finally, CA1 projects project back to the entorhinal cortex, completing the loop (Stepan et al., 2015). The trisynaptic transmission is indirectly regulated by inhibitory interneurons (Sirvio et al., 1996).



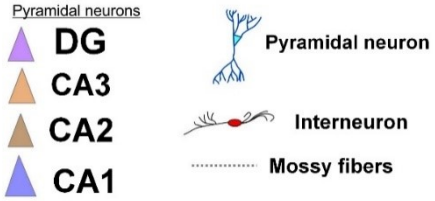


Fig 4: Scheme of a trisynaptic circuit. *Neurons in dentate gyrus (DG) receive information from the entorhinal cortex by the Perforant pathway. DG granule cells project to CA3 by mossy fibers. CA1 is than connected with CA3 by Schaffer collaterals. The loop is closed by projections from CA1 to the entorhinal cortex. Circuit transmission is indirectly regulated by inhibitory interneurons.*

In cross section of the hippocampus, several layers will be shown. CA3 consist of these layers- lacunosum-moleculare, radiatum, lucidum, oriens and pyramidal. CA1 and CA2 have the same layers except the lucidum layer.

- ***Pyramidal layer*** (*stratum pyramidale*) consists primarily of pyramidal cells which are principal excitatory neurons in the hippocampus, but there are also other neuronal types such as interneurons, bistratified cells, or radial trilaminar cells. By the naked eye, it is the most visible layer in the hippocampus, and pyramidal cells are tightly packed in the CA1, so it makes it easier to distinguish CA1 from CA2 and CA3 regions (Witter, 2012).
- ***Stratum oriens*** is a narrow layer, located deep to the pyramidal layer with a small density of neurons. There are basket cells, horizontal trilaminar cells, and basal dendrites of pyramidal cells (Maccaferri, 2005).

- ***Stratum lucidum*** is a narrow acellular zone in CA3, superior to the pyramidal cell layer. It is one of the thinnest layers in the hippocampus and the mossy fibers, which end in pyramidal layer of CA3, pass by this layer and building a border between CA3 and CA2 regions. (Nguyen et al., 1996).
- ***Stratum radiatum*** is superficial to stratum lucidum in CA3 and immediately above the pyramidal cells layer in the CA2 and CA1. It contains a majority of CA3 associational connections and Shaffer collaterals, which connect the CA3 to CA1. (Witter & Amaral, 2004)
- ***Stratum lacunosum*** and ***stratum moleculare*** are the most superficial layers of the hippocampus. There are perforant path fibers from the superficial layers of the entorhinal cortex, Shaffer collateral fibbers, and afferent connections from the thalamus. These two layers are often summarized to one layer called *stratum lacunosum moleculare*. (Förster et al., 2006)

1.3 The main neuronal types in the hippocampus

There are multiple ways how to distinguish a neuronal type- by neurotransmitters, by functional properties (inhibitory (usually interneurons) neurons or excitatory neurons (principle cells)), by their electrophysiological properties, by molecular characteristics of their membranes, by their localization, etc. Despite all of the possible approaches of diversification and neuronal types, I will describe just the pyramidal cells and basket cells (as interneurons), since they are the most represented neuronal types in the hippocampus.

1.3.1 Pyramidal cells.

Pyramidal cells are principal cells in the hippocampus, as in the whole cerebral cortex. They are projection neurons – it means that they often send their axons to long distances (Bekkers, 2011). Their basal dendritic tree extends into stratum oriens and the apical one occupies stratum radiatum. Dendritic length and organization of pyramidal cells in the CA3 region are heterogenous, in contrast to pyramidal cells in the CA1 region, which are quite homogenous. Not just the organization but also body size is different - pyramidal cells in CA1 have smaller cell bodies than those in CA3 (Hayashi & Ishizuka, 1995) (Pyapali et al., 1998). By the dendritic morphology, pyramidal cells in CA1 can be separated into two groups: cells in which apical dendrites bifurcate in stratum radiatum or cells with a bifurcation in stratum lacunosum moleculare. (Bannister & Larkman, 1995). Pyramidal cells are the main excitatory neurons and their major neurotransmitter is glutamate. Their electrophysiological properties will be described later in the thesis.

1.3.2 Basket cells

Basket cells are inhibitory GABAergic interneurons in the neocortex (Curley & Lewis, 2012), cerebellum (Southan & Robertson, 1998), and hippocampus (Gulyás et al., 2010). They synchronize the firing of pyramidal cells by perisomatic inhibition (targeting cell bodies, proximal segments of dendrites, and initial segments of axons) (Bartos & Elgueta, 2012). One basket cell carries, on average, 1500-2000 synapses with pyramidal cells (Freund & Kali, 2008). By so many connections they play a crucial role in the synchronization of firing patterns of the pyramidal cells, that can be recorded as a neural oscillation.

1.4 Function of the hippocampus

The hippocampus seems to play a role in various mental processes such as social behavior, response inhibition, anxiety, flexible cognition (and with that connected decision making), and many other cognitive mechanisms. Many processes are derived from the complex neuronal circuits in which the hippocampus is participated in. But in this thesis, I will describe two main functions of the hippocampus - memory and spatial navigation (Rubin et al., 2014).

1.5 Memory

The hippocampus, together with associated medial temporal lobes is a major system for memory. Since 1957, by the case of the HM patient with a bilateral lesion of the hippocampus (Scoville & Milner, 1957), it is well known that the bilateral absence of this structure causes a heavy anterograde and partial retrograde amnesia. This finding started a huge amount of lesion studies on the animals, to specify which type of memory is hippocampus exactly involved in and how the hippocampus arranges a consolidation of the memories (Milner, 1965) (Zola-Morgan & Squire, 1986) (Aggleton et al., 1986). Thanks to these studies, there was found a connection between spatial navigation and the hippocampus, in the long term and short term memory testing task, and the role of the hippocampus in episodic memory (Chozick, 1983).

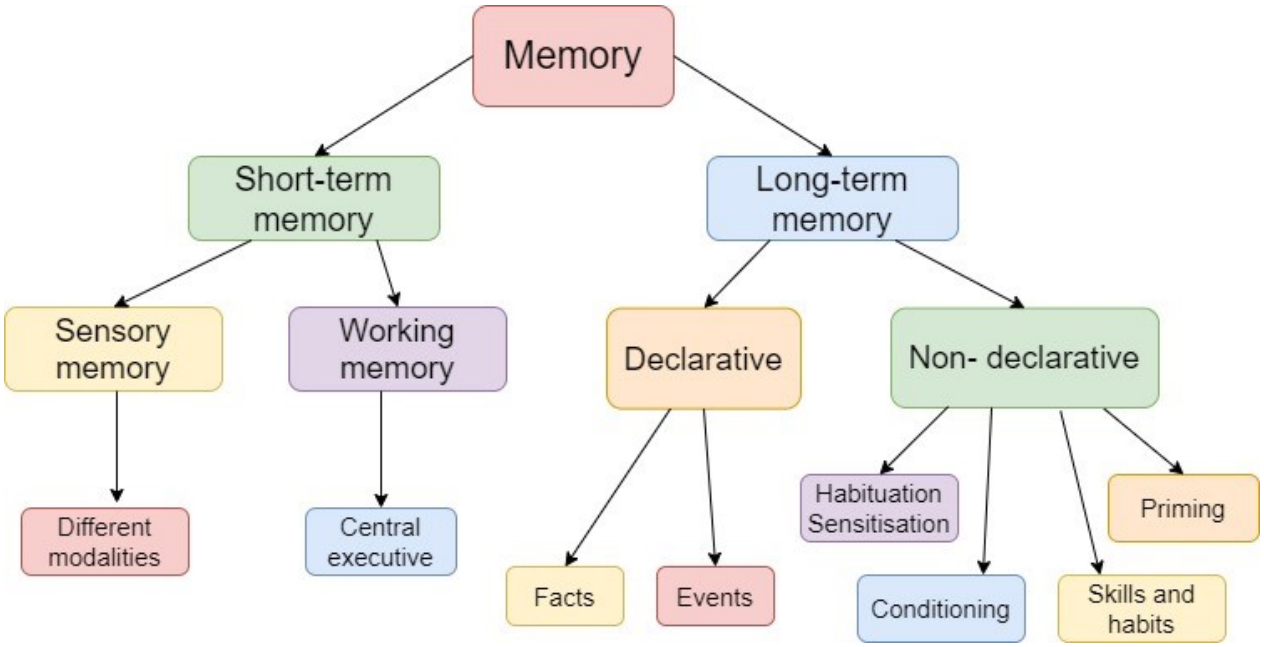


Fig 5: Schematic overview of the memory types.

Episodic memory is closely connected to the semantic memory. The semantic memory is the memory of facts, which, when attached to a context, form episodic memory (Menon et al., 2002). Both are summarized in the term declarative memory; it means that it can be declared, in humans mostly verbally. But in the case of hippocampal lesions, there is just mild damage to the semantic memories, but more evident in the episode memory, so it seems that the hippocampus is crucial mainly for the episodic memory and some authors even suggest that for brain semantic memory and episodic memory are two different systems (Tulving & Markowitsch, 1998).

1.6 Spatial navigation is also memory

According to the cognitive map theory, spatial navigation is also somehow a part of memory. Recognizing objects, learning a change in landmarks associated with rewards or nests, remembering the routes, and many more strategies necessary for spatial navigation involve a strong memory component (Eichenbaum, 2017). It has been suggested that the semantic memory is linked to allocentric (map-based) navigation since this type of navigation is dependent on cues from the outside world, which need to be consolidated for retrieval of a certain environment (Eichenbaum et al., 1999) (Buzsáki, 2005). In contrast, episodic-like memory seems to be associated with egocentric navigation (Fig.1), because for this type of navigation it is crucial to recall memories from first-person perspective and experiences (Burgess et al., 2002). Path integration strategy (see above) is then a contextual combination of individuals time and space (Kraus et

al., 2013). This theory suggests that the same neural network is responsible for both the physical and mental forms of travel. Buzsáki even suggests, that neuronal mechanism underlaying in the spatial navigation (place cells, grid cells - see below) are the system behind the mechanism of remembering, consolidation, and recalling of spatial memory and memories linked to certain environment (Buzsáki & Moser, 2013).

4. HIPPOCAMPAL ROLE IN SPATIAL NAVIGATION TASKS

Spatial cognition can be studied by various spatial tasks. These tasks are crucial for clarifying neuronal mechanisms standing behind the spatial behavior. For the testing are used specialized apparatuses called mazes. Depending on the type of task, there are cues inside, or outside the mazes, that help to understand the cognition of the landmarks in the environment of the tested subjects. I will briefly describe some of the tasks, that are fundamental in behavioral neuroscience research, and in the end, I will focus on the tasks relevant to the topic of this thesis.

1.7 Navigational tasks

Navigational tasks test the animal's ability to navigate between different places (Whishaw & Mittleman, 1986). In many of them, the animals are navigating to the hidden goal, whose position can be found in relation to the cues located outside or inside the maze. However, the final analysis of the data in some of the navigational tasks can be problematic since it is uncertain whether the animal is navigating by the egocentric or allocentric navigation.

1.7.1 T-maze

T-maze (or the Y-maze variant) is an elevated apparatus in the shape of T or Y. There are three arms, the base arm, where the animal is usually placed at the starting position and is allowed to choose between the other two arms (left or right). T-maze´s most common purpose of use, because of its simplicity, is as a testing apparatus for spontaneous alternation (Lalonde, 2002). It is a tendency to choose the arm not visited before as a reflection of the first choice. Complementary to spontaneous alternation is rewarded alternation, where the spontaneous tendency is suppressed by the reward. Rewarded alternation can test working memory, or, in the case of multiple days testing, long term memory. This can be combined with visual or odor cues and by using reversal shifts, it can test cognitive flexibility. All the mentioned tasks are hippocampal-dependent (Deacon & Rawlins, 2006).

1.7.2 Radial maze

This apparatus resembles the T-maze. The radial maze consists of eight or more arms originating from the central area. At the end of the arms, there are cups (Olton et al., 1977). In the working memory testing variant, all of the cups contain reward, thus the animal needs to remember, which arms were already visited, and the reward obtained. In the long-term memory variant of the testing, there is only one (in some versions more than one) rewarded cup, so the animal must remember, usually by the visual cues outside the maze, which arm is the correct, rewarding one (Olton, 1987).

There are many variants of the radial maze tasks. The disadvantage of the radial maze is difficult to determine if the used type of information (such as visual cue) animal used to solve the task because the rat can distinguish the correct arm also by the typical odor of the arm, even though the experimenters effort to clean the arms (Burešová & Bureš, 1981). This problem can be solved by rotation of the maze between trials (Liu & Bilkey, 1998) or the maze can be filled with water (Burešová et al., 1985).

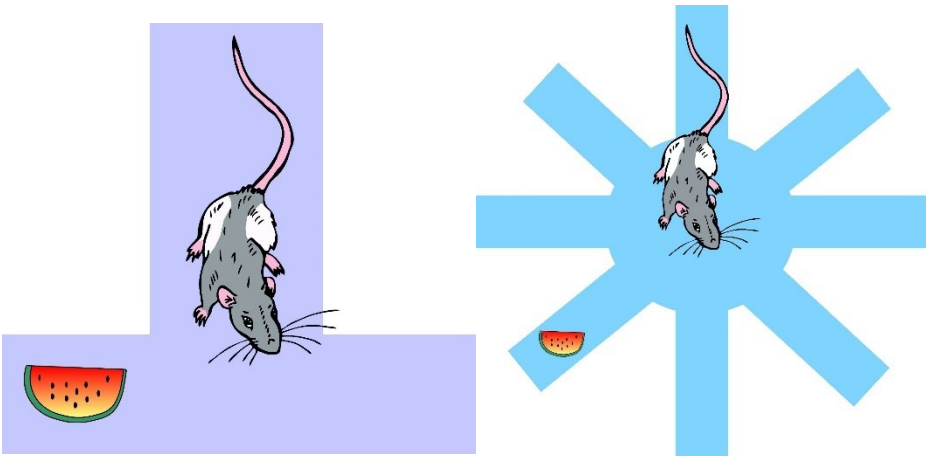


Fig. 6a: Scheme of T-maze and radial maze. *Rat are trained to visit particular arm(s) of the maze. In the case of T-maze, there are only two choices, contrary to the radial maze, which possessed of multiple choices.*

1.7.3 Morris water maze

Previously mentioned problems of unwanted olfactory navigation in the maze are eliminated in the task called Morris water maze task (Morris, 1981) (Morris et al., 1982) (Morris, 1984). An animal is placed into a circular pool (usually about 2 m in diameter) filled with colored water, so the animal cannot see a hidden escape platform placed somewhere in the pool. In most variants of the task, the animal orients by the visible cues outside the pool (such as doors or windows in the room). There are multiple variants of the Morris water maze task, but all of them test working or long-term memory (Vorhees & Williams, 2006). Hippocampal lesions disrupt the learning of the task, but once the location of the platform is consolidated,

the afterward lesions of the hippocampus had only a mild impact on successfully solving the tasks (Redish & Touretzky, 1998).

The disadvantage of this task is that the animal has to be a good swimmer and swimming should not be too stressful for it (not to affect the learning). That is why rats are, thanks to their natural habitats, proper testing subjects (reviewed in (D'Hooge & De Deyn, 2001)).

1.7.4 Carousel maze

Carousel maze is an arena developed in our laboratory (Bures et al., 1997). One of the tasks in this maze is an active place avoidance task, where the rats get a mild electric shock whenever they enter the to-be-avoided unmarked sector on the arena. The arena is continuously rotating, but the sector remains in the same place relative to room coordinates. The task taxes the ability to distinguish between two reference frames – rotating arena and stable environment outside (room-frame) (Kubík & Fenton, 2005) (Svoboda et al., 2015).

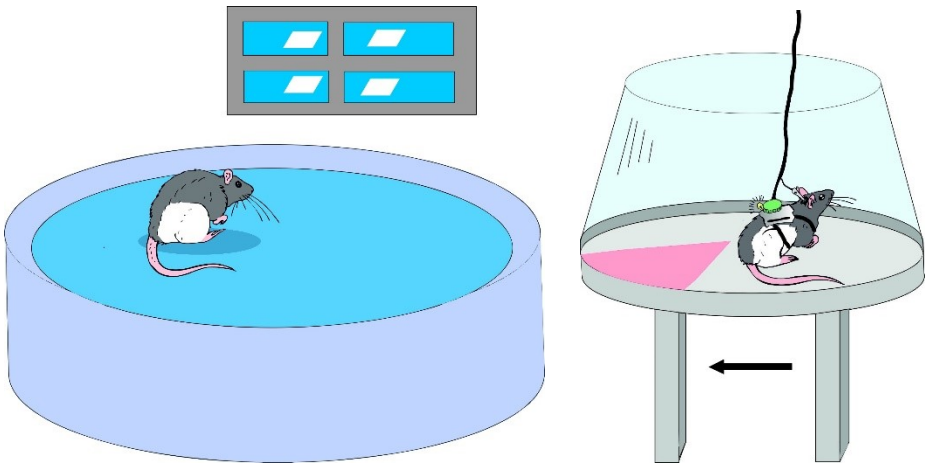


Fig. 6b: Schematic picture of The Morris water maze *(left)* **and Carousel maze** *(right). Rats can navigate due to cues in the room, outside of the maze, such as windows, doors, or shelves. Platform and the avoided sector are marked on the scheme but in real environment they are invisible.*

1.7.5 Object- position recognition tasks

These task works on the fact, that animals spend more time exploring new position of the objects, than the older one, this task tests their memory and ability to decide, whether the object position was already presented to them (Antunes & Biala, 2012). In the first phase, two identical objects are placed at two positions and allowed for an animal to explore them. In the second phase, one of the objects is moved to a novel position. The animal should recognize that the position of the objects was changed, and it should

prefer to explore the object at the novel location. A more complicated version of the task works with more objects and changing or switching their positions (Kenney et al., 2011).

1.7.6 Object position recognition using touch-screen presentation

Tasks for studying cognition in rats, which use a computer screen for the presentation of the visual stimuli, become more and more popular. Using this method has many advantages such as the possibility to present various stimuli and automated presentations. Furthermore, neuronal mechanisms underlying the cognition necessary for the solving of the spatial tasks with the screen is similar to the real environment tasks (Talpos et al., 2010).

1.7.6.1 Paired-associate learning task

This task study object-in-place paired-associative learning (Bussey et al., 2012). The animals are trained to discriminate the position of an object displayed on the touchscreen. The rewarded and the non-rewarded object are the same and the only difference is in their location on the touchscreen. As a response to the correct location of the object on the screen animal obtains a food pellet (Talpos et al., 2009).

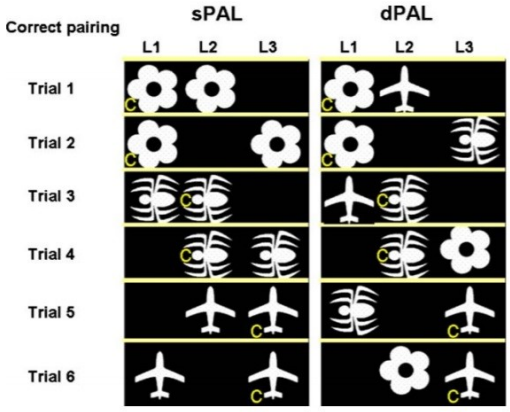


Fig 7. Object-in-place paired-associative learning task. *Example of different trials and correct pairing between objects and their location (L1-L3). The correct rewarded location for each stimulus is marked by C. From* (Talpos et al., 2009).

1.7.6.2 Trial-unique nonmatching-to-location task

In this task, animals are trained in an operant chamber to touch a rewarded area on the screen. There are two phases- sample phase and test phase. In the sample phase, visual stimuli appear in a sample location. In the following test phase, two locations were illuminated: the previous sample non rewarded location and a new rewarded location. If the rat selects the new location it is rewarded by a food pellet. This study showed that rats can utilize visual stimuli displayed on the touchscreen for spatial pattern separation. (Kim et al., 2015) (Talpos et al., 2010) Pattern separation is a process, apparently used in episodic memory, that transform highly similar sensory inputs into a distinct, dissimilar representation (van Goethem et al., 2018).

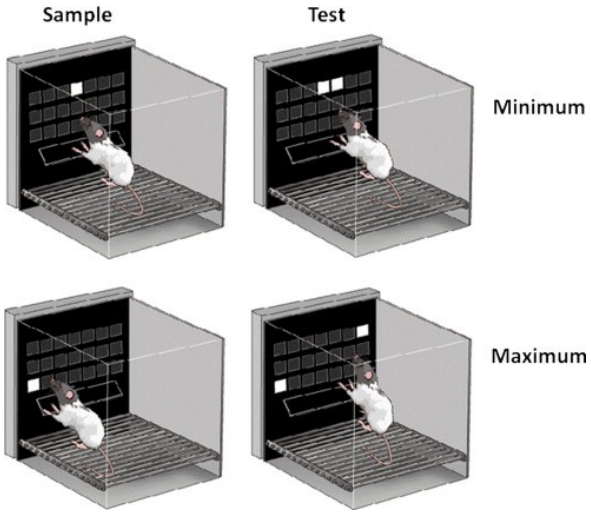


Fig. 8: Trial-unique nonmatching-to-location task apparatus. *Sample phase (left) where the visual stimuli is presented and test phase (right) which consist of already presented position and rewarded newly presented location. From (Talpos et al., 2010).*

1.7.6.3 Inaccessible object position discrimination

This task was developed in our laboratory (Laboratory of the Neurophysiology of Memory, Institute of Physiology CAS). The apparatus itself has been based on the Skinner box (Skinner, 1938). Rats are trained to press a lever for obtaining a reward when a rewarded position of the same white object (rectangle, circle) is presented on a computer black screen. (Klement et al., 2010) (Levcik et al., 2013). This apparatus is used in this diploma thesis and it will be described in more detail in the experimental part of the thesis.

Fig. 9: Schema of the apparatus for inaccessible object position discrimination. *Rat is in the box with the lever and feeder into which food is delivered when the animal executes a correct operant response (left). The operant box is in front of the monitor, so the animal has a non-obstructed view of the screen (right).*

5. THE NEURAL BASIS OF SPATIAL REPRESENTATION IN RODENTS

1.8 Place cells

During O´Keefe and Dostrovsky´s research, which was focused on the connection between the hippocampus and spatial navigation, a special type of the neurons was discovered, which fired by complex spiking dependent on rats location in the environment (O'Keefe & Dostrovsky, 1971), these cells were later morphologically defined as pyramidal cells (Henze et al., 2000). These neurons were called place cells since they are active whenever the animal enters a specific place in the environment. Each place cell represents a part of the environment known as place field, by its spatially-selective firing properties. Together, they work as a neural map of the environment. Place fields are allocentric, meaning that they are defined by the outside world rather than the body. Place cells were shown to sometimes have multiple place fields, depending on individuals' previous experience with multiple environments with various sizes (Battaglia & Treves, 1998).

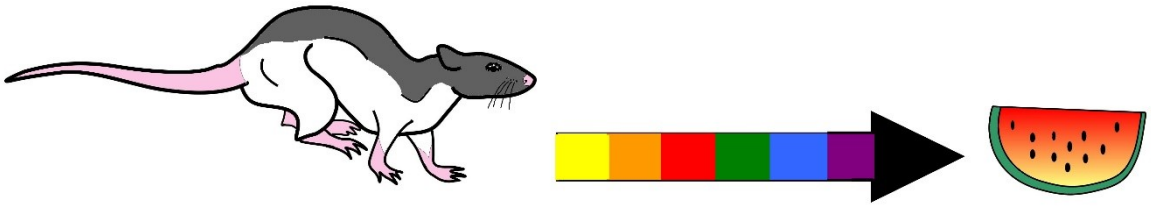


Fig. 10: Place field scheme. *Each colored square in the rat's path to watermelon represents place field of one place cell, which fires when the animal enters to this particular place.*

Place cells are part of a system that provides a cognitive representation of the space, previously mentioned cognitive map (Tolman, 1948). Place cells are located mainly in the CA1 and CA3 fields of the hippocampus proper and in Gyrus dentate (see the chapter "hippocampus anatomy") (Park et al., 2011). A higher density of these neurons is in the dorsal part of the hippocampus, but they were also observed in the ventral part (Poucet et al., 1994), despite having broader spatial tuning and other characteristics. The number of spikes fired by the place cell at a specific place encodes information about the animal position. This spatially selective increase in firing rate is the hippocampal rate code.

Place cells are flexible, and they can suddenly change their firing patterns, this phenomenon is called remapping. Remapping can be global, or partial. In the case of global remapping, most of the cells remap, change, or lose their place field location. Partial remapping affects just a small portion of the place cells, the rest of them remains intact (Latuske et al., 2018). These procedures can be caused by changes in

environment like shape and size, colors (Jeffery et al., 2004) or conditional cues (like odors) (Allen et al., 2012). As mentioned earlier, the activity of someplace cells is influenced by the previous or future location of the animal (Wood et al., 2000). This suggests that the firing can be affected by the previous experience and there is a relation between place field and episodic memory.

Place cells can be reactivated, outside of their place field and this reactivation is usually faster. During sleep, reactivation is in the same order as a previously experienced behavioral trajectory (Ji & Wilson, 2007), but during awake state (mostly during the period of relative immobility) place cells are reactivated usually in the reversed order(Foster & Wilson, 2006). This process is called replay and it is considered to play a role in spatial memory consolidation. The same repetition sometimes occurs before the testing task, called the preplay, when animals do know that they will be exposed to the environment that they already know (Dragoi & Tonegawa, 2011). It could be a form of prediction based on previous learning.

Previously I mentioned that place cells can be found in CA1 and CA3. It was observed that the place cells from these two locations are not having the same characteristics (Lee et al., 2004). CA3 place cells are more conservative, and they are not remapping so often such as CA1 place fields (Knierim, 2002). It was found that the plasticity of CA1 place cells appears to be temporary and the place fields reset back to their initial starting locations after remapping (Knierim et al., 2006). However, their functional differences are not still fully clarified.

1.9 Grid cells

Place cells are not the only neurons, that react by their firing pattern to the environment around the animal. Other environment-based groups of cells are grid cells (Hafting et al., 2005). They are not in the hippocampus proper, as the place cells, but in the medial entorhinal cortex (the main input to hippocampus (see the hippocampal formation in previous chapters)). When the animal navigates in an open area, grid cells fire at the regular pattern. Grid cells play a role in storing and integrating information about distance, location, and direction (Hafting et al., 2005). Grid cells, in contrast to place cells, have multiple firing fields that are repeated across the environment. These fields are arranged in a regular triangular structure (Burgess et al., 2011).

Grid cells may be crucial for a basic form of navigation called path integration (Hafting et al., 2005). In this type of navigation, animal computes a distance and directions vectors on the route for easier return, during the absence of landmarks or other cues. Disruption of grid cell activity by removing NMDA

glutamate receptors from the retro-hippocampal region impairs path integration performance, without affecting other spatially-selective cells (Gil et al., 2018). These results supported a theory that grid cells are involved in spatial navigation by path integration. Grid cells became an object of research in relation to the episodic memory, but this function is beyond the problems discussed in this thesis (Hasselmo, 2009).

Are the place cells and grid cells connected? The answer is yes, most of the outputs from the entorhinal grid cells are connected to the hippocampal place cells. The biggest question about that connection, that remains as a subject of research is, how the periodic spatial firing pattern of the grid cells is transformed into a non-periodic signal in place cells? The explanation could be, that signal from grid cells is transformed, with overlapping firing fields and different orientation and spacing, by linear summation. Therefore, differential responses among the modules of the grid cells might lead to the remapping of place cells (Fuhs & Touretzky, 2006) (McNaughton et al., 2006) (Solstad et al., 2006).

1.10 Head direction cells

Head direction (HD) cells are neurons, that increase their activity when the animal points its head in certain directions (Taube et al. 1990). Neurons with this specific activity were found in many different brain regions such as the entorhinal cortex (Sargolini et al., 2006), thalamus (Goodridge & Taube, 1997), striatum (Mizumori et al., 2000) or mammillary nucleus (Stackman & Taube, 1998). They are dependent also on the vestibular system, which is an otolithic organ responsible for providing sensory information of the orientation, rotation, and balance of the head. The activity of the HD cells is allocentric, it means that are not dependent on the changes of the environment or either position of the rest of the body relative to the head. Different HD cells show different preferences for the directions (Taube, 2007). Altogether they cover the entire compass. This compass works just for two dimensions (horizontal view) and there are theories that HD cells are interconnected to the imaginary circle and each cell codes its own or neighboring directions and suppressing other not relative directions. This theory is called “Ring attractor theory” (Laurens & Angelaki, 2018). But there were not found yet enough interconnections between HD cells or anatomical organizations, supporting this theory.

1.11 Boundary cells

Boundary cells (also known as border cells) are neurons found in the hippocampal formation and they respond to the presence of the environmental boundary in a particular distance at the receptive field in a specific allocentric direction from an animal. They are connected to the place cells and they contribute

with environmental information to place cell firing (Lever et al., 2009). It was also suggested, that grid cells integrate information about borders and their distance relations (Krupic et al., 2016).

1.12 Object vector cells

Object vector cells are neurons located in the medial entorhinal cortex that fire at given distances and directions from spatially confined objects. They might provide a cellular basis for positional mapping of the space between objects. It is a newly discovered type of neurons, that surely plays an important role in space coding, but their functional properties and connectivity needs to be more clarified (Høydal et al., 2019). Object vector cells represent position of objects in accessible space, however, our experiment is designed for objects located in inaccessible space.

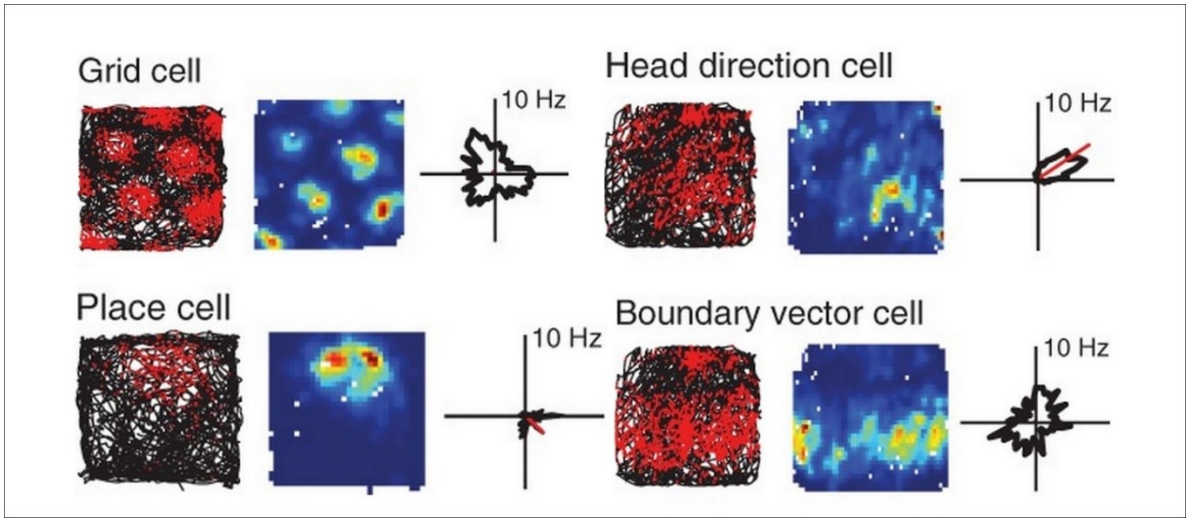


Fig. 11: Spatial related activity differences between grid cells, head direction cell, place cell and boundary vector cell during Open field task*. Rat is allowed to move freely in the monitored box, during electrophysiological recording. First picture represents the trajectory of the rat, where activity of cells is colored by red color. Second picture is histogram of neuronal activity in the observer space and third picture represents the cellular activity during different angles of head movement. Simplified picture from (Brandon et al., 2014).*

6. ELECTROPHYSIOLOGY

1.13 Electrophysiology as a tool to understanding brain

Electrophysiology is a branch of physiology, that studies electrical properties of cells, in our case neurons. Since the function of the brain is based on the electrical activity of neurons, electrophysiological techniques are crucial in neuroscience research. In contrast to optical or genomic methods,

electrophysiological methods dispone by high time resolution (in terms of milliseconds) at expense of spatial resolution.

Electrophysiological techniques can be separated into two groups- intracellular recording (Voltage clamp, Current clamp, Patch-clamp) and extracellular recording. Intracellular recording provides information about single-cell physiology and they are usually used *in vitro*. This is the reason why this thesis won't mention them anymore because our experiments were carried out in behaving animals.

Extracellular electrophysiological multi-unit techniques can record the activity of many (hundreds or thousands) neurons simultaneously. Multi-electrode arrays, with a small diameter can record the single-neuron activity and local field potential (LFP). LFP is an electric potential in extracellular space around neurons (Buzsáki et al., 2012). For this experiment, we used tetrodes composed of very thin wires (see in methodic) which can be used for measuring not just LFP but also single-neuron activity nearby the tetrode.

1.14 Electrophysiological properties of neurons

By their firing pattern, neurons can be categorized as regular spiking (RS), intrinsically bursting (IB), chattering neurons (CH), and fast-spiking neurons (FS) (Wijekoon & Dudek, 2007). RS type is the most typical type in the cortex - after the presentation of prolonged stimulus neurons fire a few spikes with short inter-spike period and then the period increases (this phenomenon is called the spike frequency adaptation. In other words, their action potentials are followed by hyperpolarizing afterpotentials (Gray & McCormick, 1996). IB neurons respond to threshold pulses with a stereotypical burst of spikes (usually two to five) followed by repetitive single spike and CH neurons can fire stereotypical bursts of closely spaced spikes (Izhikevich, 2003). The last class of neurons is FS. This class consists of inhibitory interneurons with short-duration of action potential and displaying little (or none) spike-frequency adaptation during short depolarizing current pulses (Descalzo et al., 2005). For more details see (Izhikevich, 2003).

1.15 Hippocampal oscillatory activity

In the hippocampus, there are three major network patterns- theta oscillations, sharp waves (and associated ripples), and gamma oscillations.

1.15.1 Theta oscillations

Theta oscillation is synchronization activity in the range of 4-7 Hz, which arises as a result of currents of neurons, dendritic calcium spikes, and other voltage-dependent membrane oscillations (Kirk & Mackay, 2003) (Aarne Ylinen et al., 1995). This oscillation is modulated by the perisomatic inhibition of pyramidal cells by interneurons (Losonczy et al., 2010). Important role of theta oscillation in spatial navigation relates

to phenomenon called theta phase precession. When the rat enters the place field of a certain place cell, the initial spikes arise to the late phase of the theta cycle. As the rat passes through this place field, the phase gets to its maximal amplitude. When animal leaves the place activity decrease back to its minimal amplitude. (Skaggs et al., 1996) (Yamaguchi et al., 2002) (Jeewajee et al., 2014).

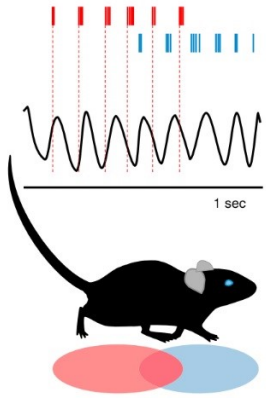


Fig 12: Schematic picture of theta precession. *The activity of place cells in their place fields corresponds to the phase of theta rhythm in dependence on the center of the activation field. This effect is known as the Theta phase precession. From* (Marozzi & Jeffery, 2012).

The role of theta oscillation in spatial navigation was already discussed, but these oscillatory activities of the hippocampus are also investigated in the connection with memory. It was found, that generating this rhythmic activity can support memory consolidation (Backus et al., 2016) and it plays a critical role in the induction of long-term plasticity, which is associated with episodic and semantic memory (Buzsáki, 2005).

1.15.2 Gamma oscillation

Gamma oscillations reflect frequencies between 40 and 150 Hz (Colgin et al., 2009). They are presented in all brain structure with fast inhibition provided by soma-targeting interneurons (Freund, 2003). Gamma oscillations are generated between principal cells and interneurons. In the hippocampus, these oscillations can be coordinated by long-range interneurons or excitatory connections toward the whole hippocampus (Buzsáki & Wang, 2012). Generally, gamma range oscillations occur whenever is important to synchronize neurons for rapid operation. This process is sensitive to filter unnecessary inputs and this is under the control of interneurons (Colgin & Moser, 2010). Retrieving of memories, attention selection processes and memory encoding are one of many suggested processes relying on the gamma oscillations (Montgomery & Buzsáki, 2007) (Muzzio et al., 2009) (Colgin & Moser, 2010).

1.15.3 Sharp waves and ripples

In the state of immobility or during sleep, theta oscillations are replaced by large- amplitude field potentials called sharp waves. They are initiated by a self-organized population of burst derived by pyramidal cells from CA3. Projection of these cells to CA1 is causing another electrophysiological phenomenon called ripples generated by CA1 pyramidal cells (Fernández-Ruiz et al., 2019). These fast field

oscillations that reach from 140 to 200 Hz, targeting parahippocampal structures, are suggested to have a causal role in memory consolidation during sleep (Girardeau & Zugaro, 2011).

1.16 Hippocampal CA1 as a crucial region in spatial navigation

As mentioned before CA1 is a region, which contains most place cells, and by that, it makes CA1 crucial in spatial navigation. There are two main groups of electrophysiologically distinguished neurons - pyramidal cells and interneurons. The anatomical properties of this region, synaptic circuits, and properties of pyramidal cells were described in detail in previous chapters.

1.16.1 Pyramidal cells

CA1 pyramidal cells receive excitatory (glutamatergic) as well as inhibitory (GABAergic) inputs from presynaptic neurons. Just around 5% of synapses are inhibitory inputs, resting 95% is excitatory (Megías et al., 2001). The main excitatory inputs arrive from CA3 and the entorhinal cortex. Pyramidal CA1 neurons have a resting potential, measured in slice preparation, between -60 to -70 mV (Staff & Spruston, 2003). Characteristic firing pattern of the hippocampal pyramidal cells are complex spike bursts, therefore they are called complex-spike neurons (Fox & Ranck, 1981). Pyramidal cells in CA1 change their firing patterns during spatial navigation. Their oscillation frequency is determined by animals traveling velocity. This makes them, from a physiological perspective, speed-dependent oscillators (Geisler et al., 2007).

1.16.2 Theta cells

The second important type of cell is inhibitory interneuron. One interneuron can target thousands of pyramidal cells(Cobb et al., 1995). During exploration, their activity increases with a speed and it is modulated by the oscillatory theta rhythm. Grace to this effect they acquired another name- theta cells(Ego-Stengel & Wilson, 2007). They are characterized by their rhythmic firing activity and shorter action potentials in comparison with complex-spike neurons (Thomas et al., 1998).

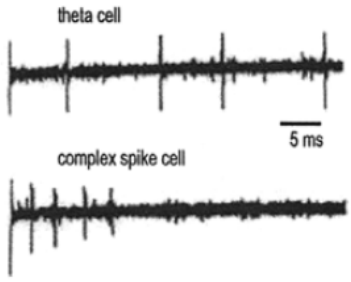


Fig. 13. Difference between complex-spike cells and theta cells. *Complex-spike cells (pyramidal cells in the hippocampus) are characterized by complex spike bursts in which the later action potential in bust has a lower amplitude. Whereas theta cells have a steady firing rate and constant amplitude spikes (Amaral et al.,2007).*

Hippocampal pyramidal cells, with their complex spike characteristics, are a crucial neuronal mechanism underlying the spatial behavior. In the practical part, we will investigate their activity pattern during discrimination of the positions of inaccessible object.

EXPERIMENTAL PART

7. AIMS OF WORK

Pyramidal cells, located in CA1 part of the hippocampus, are important neurons responsible for spatial navigation. Many studies investigated their connection to objects' positions discrimination, but there are no studies, that clearly describe their role in discrimination of objects' positions in inaccessible space. For this purpose, we used a task, that provided us with the possibility to train rats to discriminate these types of objects' positions. By using electrophysiological methods, we can measure the activity of individual cells in the hippocampus during the task. This experiment may clarify a role of hippocampal pyramidal cells in coding of positions of inaccessible objects.

The first aim is to design the inaccessible object-position discrimination task using two different reward positions. Two types of configurations will be presented to animals and the main objective is to decide, which type of configuration is easily learnt to discriminate.

Second aim is to show whether and how information about the position of inaccessible objects is represented in the hippocampus using single-neuron electrophysiology.

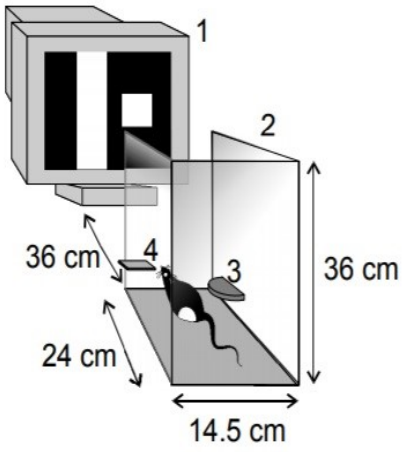
8. MATERIALS AND METHODS

1.17 Subjects

We used adult male outbred Long–Evans rats (N = 10; 3-month old at the beginning of the experiment). The rats were obtained from the breeding colony of the Institute of Physiology of the Czech Academy of Sciences (IPHYS). The breeding core was derived from Charles River (Italy), but the rats have been bred at IPHYS for multiple generations. After the delivery from the breeding unit, they were acclimated for 10 days before commencing the handling. They were housed in plastic transparent cages in pairs before the surgery and individually after surgery in an accredited temperature- and humidity-controlled air-conditioned room with a 12/12-h regular light-dark cycle. Water was always freely available, but access to food was restricted to maintain the rats at 90% of their free-feeding weight. Animal welfare complied with current legislation (the Animal Protection Code of the Czech Republic and EU Directive 2010/63/EC). The experiments were approved by the local and Ministry animal care committees (Project of experiments 50/2016). Before behavioral sessions, animals were handled for 5 days per 10 minutes and they were placed to the experimental room for one hour to adapt. One of the animals suddenly died before surgery without any symptoms of disease or visible causes. After completion of the behavioral study, they transcardially perfused in deep ketamine/xylazine mixed anesthesia for future histological and immunohistochemical analysis.

1.18 Behavioral apparatus

The apparatus consisted of an operant chamber (Fig. 14b), a feeder, an LCD monitor located 37 cm in front of the chamber, and a computer (Fig. 14a). The reward (45-mg chocolate pellet; Bio-Serv, USA) was delivered from the feeder after a rewarded response. The computer registered lever presses activated the feeder and synchronized the events and presentations of stimuli on the screen with neural data recording. The custom-based software for stimulus presentation was written in Python by an external collaborator.



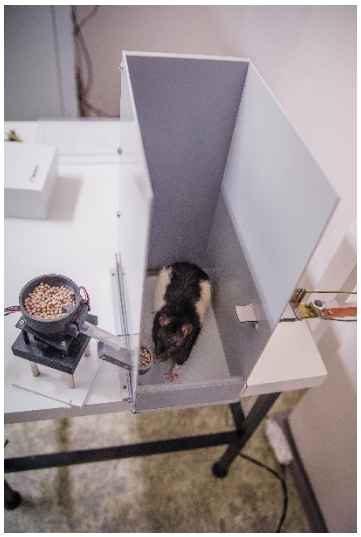
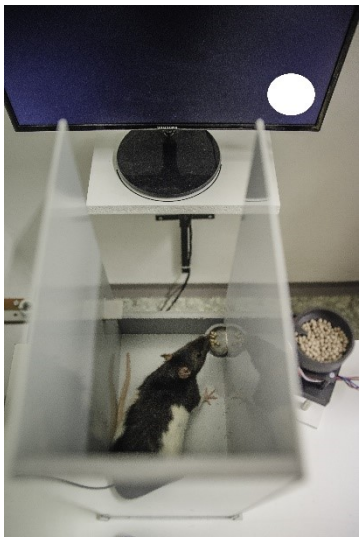



Fig.14a :Scheme of the size of an operant chamber (Nekovářová, 2006) **.Fig.14b: A photo of the apparatus during the learning session** *(photo by Kristýna Malenínská).*

1.19 Experimental design

Food-deprived rats were pre-trained to press the lever when a rewarded position was displayed on a distant computer screen. During learning, we used two groups of animals. One group (n=5) was trained in the one-dimensional object-position discrimination task, in which the object (a white rectangle) changed its positions only in terms of the X-axis and the second group (n=4) was trained in the two-dimensional object-position discrimination task, in which the object (a white circle) changed its position in x and y axis (see Fig. 15.). Two rewarded and two non-rewarded positions were presented over the course of training. Individual stimulus presentations were separated by a black screen presentation of a duration of 3s. Only one of the stimuli was presented at the time. We used four different configurations with increasing difficulty, e.g. the time for individual stimulus presentation (90 s in the first configuration) decreased over the course of training. In the final stage of training, each stimulus was presented for 15 seconds. A pseudorandom sequence of displayed stimuli was used to prevent the rats from adopting a time strategy for solving the task with a condition, that same stimulus was never displayed twice in sequence nor two rewarded stimuli were presented consecutively. A variable ratio for reward delivery was applied, with 3 correct presses in average to obtain a reward (and never more than 5 presses during the single presentation). Each rewarded position was displayed 18 times and each non-rewarded position was displayed 36 times, resulting in total time of approximately 32 minutes per session. As a criterium whether rats can be implanted for recording sessions was a success rate at least 60% during five consecutive sessions. The difference between the learning phase task and recording phase task was, that during recording session rewarded stimuli were presented 24 times and non-rewarded stimuli were presented

48 times in total duration of 45 minutes for session. These stimuli were separated by a 3- or 5-seconds break, so we could reduce the anticipation of the next stimulus onset.

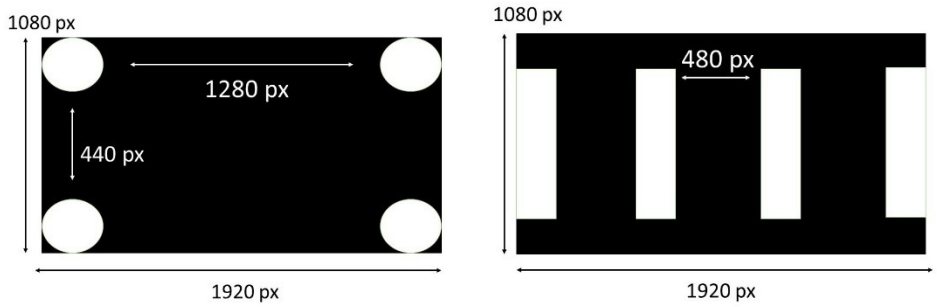


Fig.15: Types of stimuli. *Two types of stimuli were presented circles (left) and rectangles (right). The area of the screen was 1920x1080. The distance between rectangles was 480 pixels and between circles 440 and 1280 pixels.*

Rat 13	*Rectangle 1 + 3*
Rat 14	*Rectangle 1 + 3*
Rat 15	*Rectangle 2 + 4*
Rat 16	*Rectangle 2 + 4*
Rat 17	*Circle 1 + 3*
Rat 18	*Circle 1 + 3*
Rat 19	*Circle 2 + 4*
Rat 20	*Circle 2 + 4*
Rat 21	*Rectangle 1 + 3*

Fig. 16. Table of rewarded positions for each trained rat.

1.20 Surgery

Rats were treated perorally with antibiotics (Enrogal *(Enrofloxacinum)* 100mg/ml 0,5ml to 0,5l of drinkable water) five days before surgery. They were anesthetized with isoflurane mixed with air (the range between 1.7% to 3.5% depending on the depth of anesthesia and 5% for induction in the induction chamber). After the induction of anesthesia, they were mounted to the stereotaxic apparatus and a midline incision was made to expose the skull. The whole area was well cleaned and treated by betadine. Six small trephine openings for stainless steel screws were drilled. Two of them served as grounding and two reference electrodes. The main craniotomy was made above the dorsal hippocampus (AP=-4 mm, ML= -2.5 mm) according to the stereotaxic atlas (Paxinos, 2007), where the Microdrive implant was attached and the tetrodes were moved above the dorsal hippocampus (DV= 1.4 mm). Dura was gently excised before lowering the tetrodes into the brain. Movable shuttles with tetrodes were covered by medical Vaseline so that dental cement used for fixation would not prevent them to move after the surgery. The wound was

sutured if needed, and local antiseptics (Betadine, Iodisol) and anesthetics (Mesokain) were applied to prevent infection and pain. After surgery animals were treated by the mix of the same antibiotics and analgesics (Nurofen (Ibuprofen) 20mg/ml 2,5 ml per 0,5l of drinkable water) for 3 days (or more if needed). Each animal was also placed in a large plastic cage, so the implant could not be damaged. The rats were monitored daily and were left to recover from the surgery for at least 7 days.

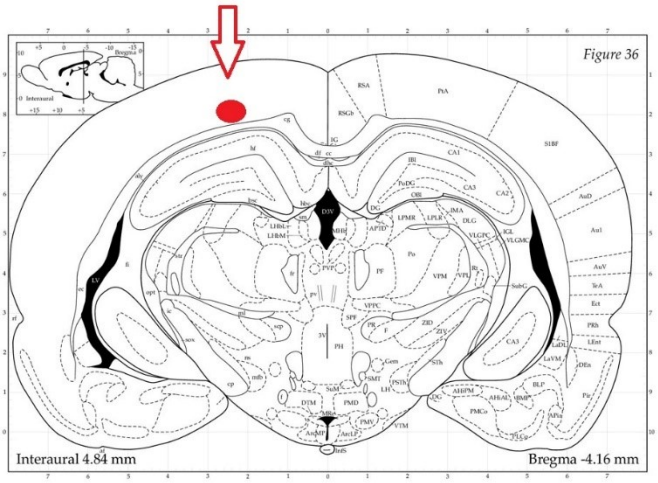


Fig. 16: Localization of the initial position of the tetrodes marked with a red dot. *Image taken from Rat stereotaxic atlas by Paxinos* (Paxinos, 2007).

1.21 Implants

For single-unit recordings, we made a tetrode from insulated nichrome wires (alloy of nickel and chromium) in a diameter of 12 µm. Each tetrode consisted of 4 wires and each of them represented an individual channel. We used a VersaDrive from Neuralynx (Fig.17) as a driver ensuring vertical movement of the tetrodes. We used 8-tetrode version of the drive to measure from 32 electrodes. After the construction of VersaDrive, a day before implantation, we gold-plated individual wires to have impedances in range 150 to 300 kOhm.

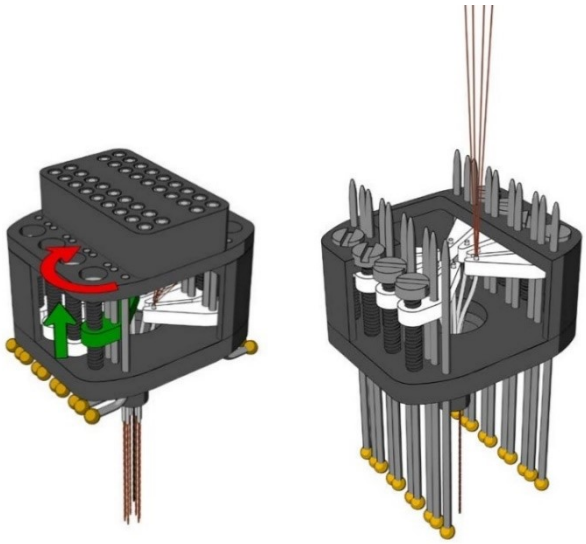


Fig.17: VersaDrive from Neuralynx*, that was used in our experiment. Tetrodes are glued to the guide tubes, which are attached to individual shuttles. Shuttles can be moved up and down separately by screws (the red arrow). Taken from official VersaDrive Manual*.

1.22 Recording techniques.

The hippocampal neurons were recorded in unrestrained animals placed in the operant chamber. Tetrodes were lowered with the VersaDrive by approximately 30-µm steps. In the beginning, to reach a hippocampus the maximum increment was 120 µm per day. Each tetrode could have been moved separately. If the single-unit hippocampal activity was present, the animal was tested in our task and electrophysiological recording was performed simultaneously. Neural data from 32 channels were amplified (1000 x) with four Lynx-8 amplifiers (Neuralynx, USA). For recording of individual spikes, the raw signal was band-pass filtered at 300-9000 Hz and sampled at 31 250 Hz with a CED Data Acquisition System (CED, UK). Two light-emitting diodes (LEDs) were present on the headstage, so the position and orientation of the head could be monitored and synchronized with the neural data recording. The positions of the LEDs were observed by an overhead camera. Events such as lever presses, pellet delivery, and the presentation of stimuli was recorded and time-stamped using the CED recording system.

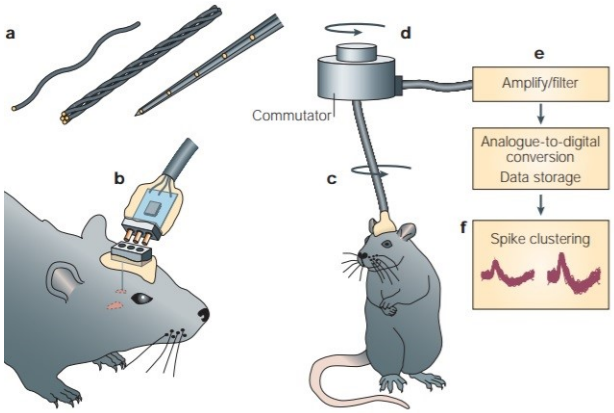


Fig. 18: Scheme of recording. *Microelectrodes are twisted to the tetrodes, which allows single neuron recording (a). Rat is connected to the head stage (b) to the system (c), that consists of amplifiers and analogue to digitals converter (from (Maren & Quirk, 2004)).*

1.23 Data analysis

Every neuron is in different distance and angle from individual wire of the tetrode, so it is possible to form the signal analyzation to distinguish single-neuron activity. This activity was analyzed by cluster analysis.

This method allows to create a graph of activity of all recorded cell, with respect to chosen parameter, with reduction of the noise and by that it makes possible to differentiate single neurons. In single tetrodes, action potentials of each cell is specific for individual channel, it means that different neurons can be assigned to individual clusters. This analysis was done by our cooperators Sergio Díez Hermano, M.Sc. and José Antonio Villacorta-Atienza (Complutense University of Madrid).

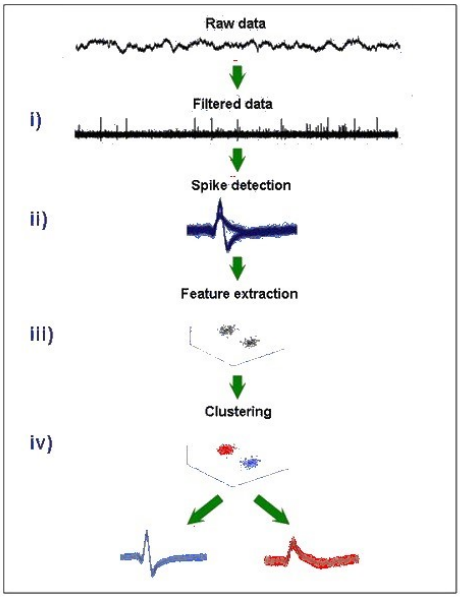
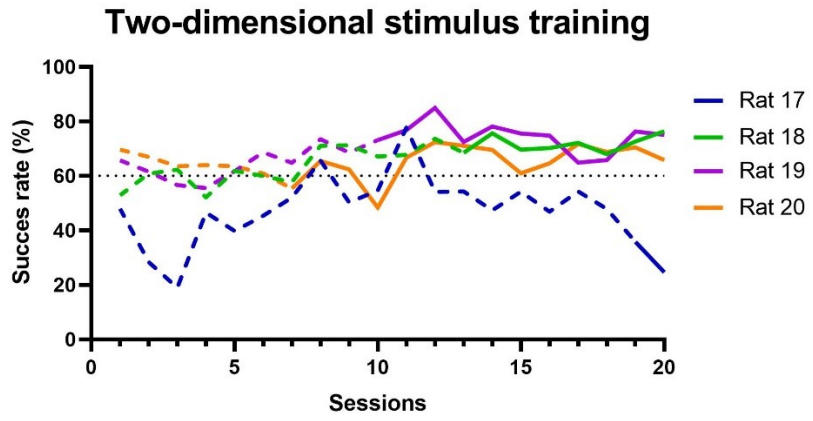


Fig. 19: Scheme of data analysis*. Raw data are filtered (i) with removal of noise. Filtered data allows to detect spikes (ii), which are than sorted to clusters (iv). Each neuron can be identified due to their action potential sorting according to chosen parameter. Adapted from (Quiroga et al., 2004).*

9. RESULTS

1.24 Learning phase

As mentioned in methods two groups of rats were tested, one with two-dimensional circle stimulus training second with one-dimensional rectangle stimulus training. Unfortunately, no rat from one-dimensional object-position discrimination group could reach a 60% of success rate, nor final configuration. From two-dimensionally trained rats just one of four rats could not reach the criteria. Only last 20 trials from the learning phase is plotted in the graph.



Tested animal	Total number of sessions
Rat 18	63
Rat 19	89
Rat 20	64

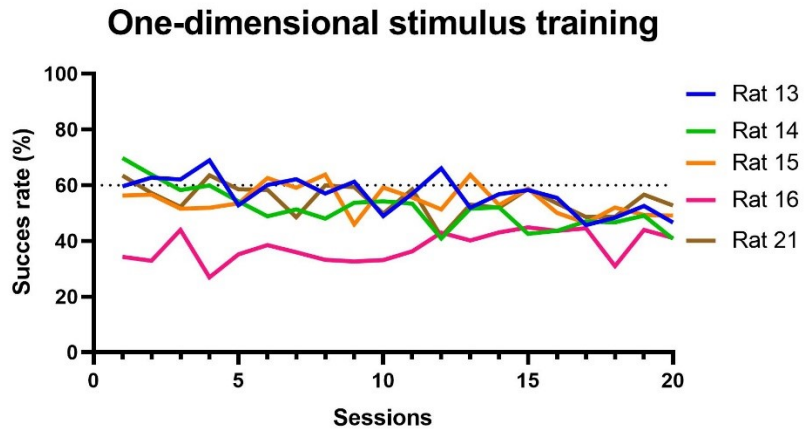


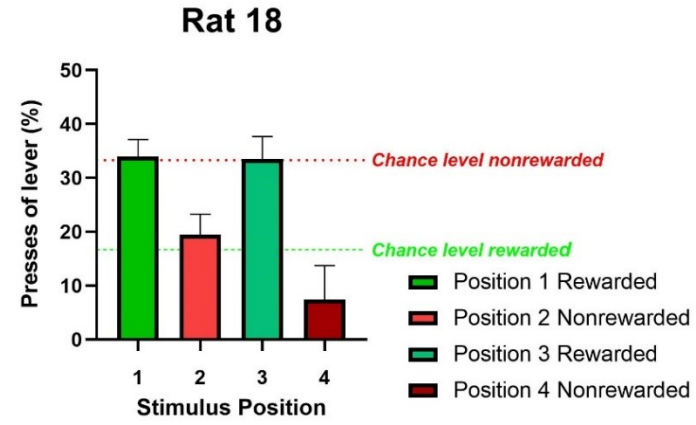
***Fig. 20: Learning phase graph**. Rats trained on circle stimulus and on rectangle stimulus. To reach a final configuration, success rate had to be above 60% during five consecutive sessions. In first graph, final configuration is marked by uninterrupted line since the animals were able to reach criteria in contrast to second graph (rectangle training) where none of the subjects was able to reach the criteria. Next to the graph, you can see a table with total number of sessions of the rats, that reached the final configuration.*

1.25 Recording phase

Data recordings from two rats are presented in this thesis. From three separated sessions from each animal, recordings with single-unit signal on different tetrodes were analyzed for the purpose of this thesis to ensure different cells were used for the analysis. Third animal was still tested in the time of this thesis.

Rats clearly discriminated rewarded positions during recording sessions. It is visible on the graph (Fig. 21) were you can see a percentage of lever presses during each position. All the presses from three recorded sessions are averaged in the graph. Since non-rewarded stimuli were presented with double frequency, chance level for non-rewarded is 33.33% and for the rewarded one 16.67%.

As mentioned before, rat 18 and rat 20 has different rewarded stimuli (Fig.14). Rewarded stimuli and their chance level is marked with green color while nonrewarded stimuli and chance level are marked by red color.



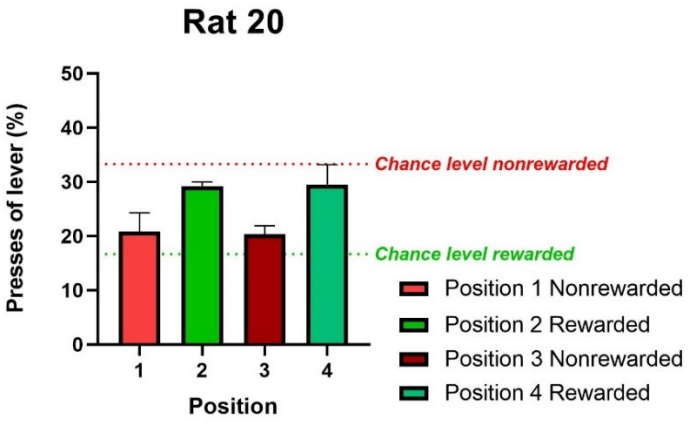


Fig. 21 Graph of percentage of lever presses during a recording. *Rat 18 and 20 had different rewarded positions. Rewarded positions are marked with green color, non-rewarded by red color. Chance levels are marked by dashed line.*

Totally 123 cells were recorded, and as you can see on graph (Fig.22a), based on the firing rate and presence of complex-spikes 91.9% of the neurons were putative pyramidal cells. And 8.1% neurons were putative theta cells. From 113 of pyramidal cells, 19.5% was responding by their firing activity to rewarded positions and 10.6% responded to non-rewarded positions. We have not identified any specific activity related to single object's position (See more at Fig. 22b).

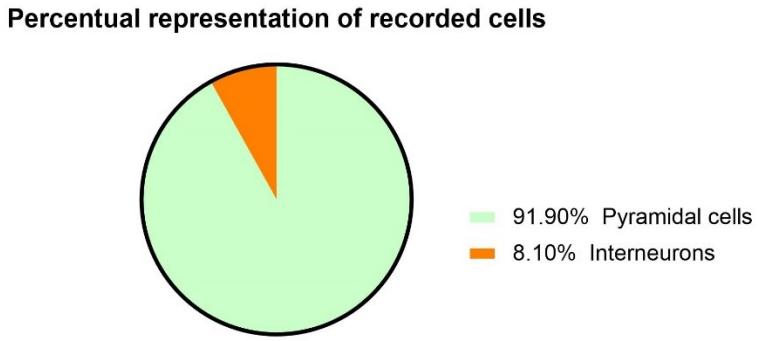


Fig.22a: Percentual representation of recorded cells. *Pyramidal cells (teal), theta cells (orange). From 123 cells, 113 had firing properties of pyramidal cells (mean firing rate was about 1.11 Hz with standard error of the mean (SEM) +- 0.09 Hz) and only 10 cells were probably interneurons (mean firing rate was 7.42 Hz with SEM+- 0.41 Hz)*.

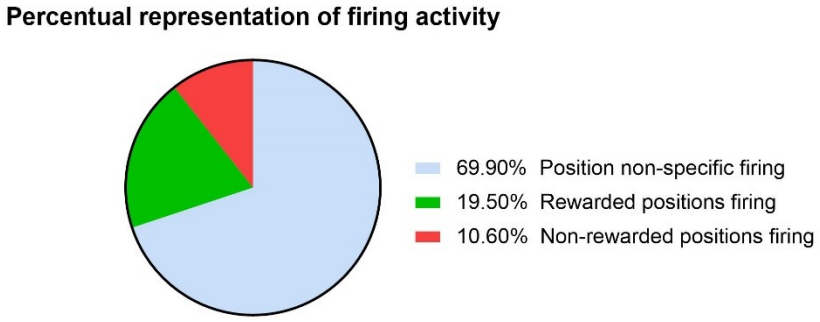


Fig.22b: Percentual representation of firing activity of pyramidal cells. *22 neurons from 113 recorded pyramidal cells (green) fired specifically during representations of rewarded positions (19.5%) and 12 neurons (red) responded by their firing activity to non-rewarded positions (10.6%). Rest of the pyramidal cells (teal) did not show object-position specific firing activity. There was no pyramidal cell, that would have a specific activity to a specific position. By specific firing we considered double of firing rate during specific positions (e.g. rewarded) than during other presented positions (e.g. non-rewarded). Also, the activity could not be connected to pressing a lever or obtaining a reward.*

The neuronal types were distinguished by their typical electrophysiological activity. As representative example from Single-neuron measurement the complex- spiking pyramidal cell in the form of raw unfiltered data, is presented here

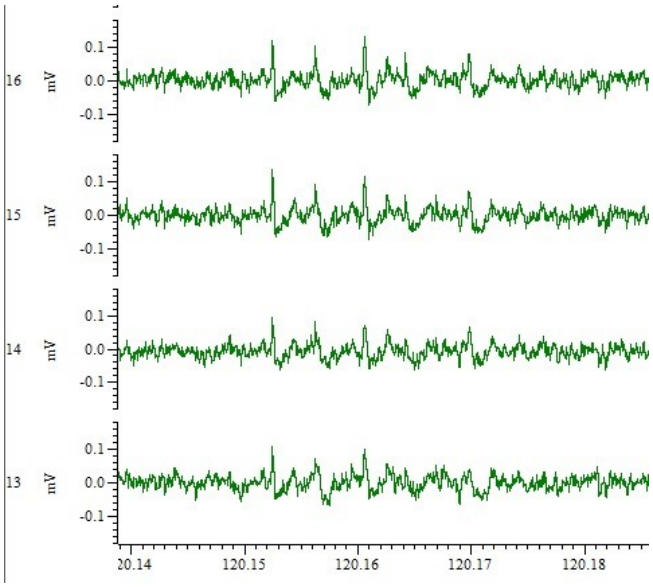


Fig. 23: Complex spiking pyramidal cell. *In this raw unfiltered recording, there is visible action potential of single neuron. It is complex spike bursting typical for pyramidal cell in CA1 region.*

From the 34 recorded pyramidal cells, that responded by their firing pattern to the object position in our task, four examples will be presented in next for pages. Red dashed line represents the stimulus onset. Time before that was filled with black screen break. Rewarded (Rew) and Non-rewarded (NonRew) positions are summarized in one graph, or separately as Stim 1-4. Lever presses histogram is shown in blue color and reward (activation of feeder) histogram in orange color. Time on the scale is in milliseconds. As you can see Figure 24 and 25 are cells responding to rewarded positions and Figure 26 and 27 are cells that respond to non-rewarded positions.

Term trial means a single presentation of the object's position and spikes per bin means number of spikes in bin, which was 100ms. In the raster plot, you can see black vertical bars, that present single spikes of the cell. With teal vertical bars, the first level press within the trial is marked (at least 1 s after stimulus onset) and with orange vertical bars, the first reward delivery within the trial.

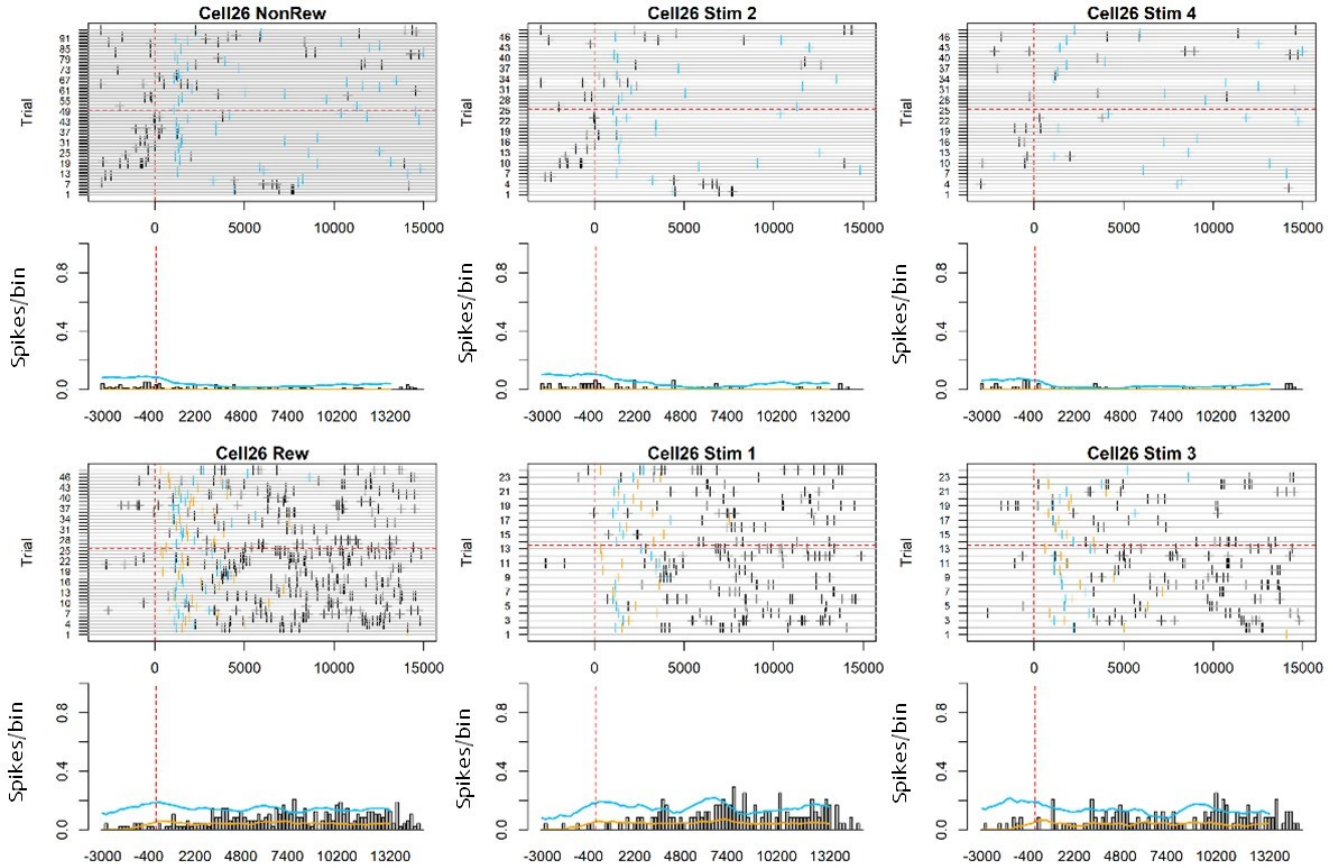


Fig.24 Cell n. 26 from rat 18. This cell, located in CA1, responded to rewarded positions. Red dashed line represents stimulus onset. The activity of neuron is increased during presentation of rewarded positions (Rew), whereas during non-rewarded positions (NonRew) there is only a weak spontaneous activity of the cell. During rewarded positions (Stim 1 and Stim3) neuron was firing with a similar probability and during each non-rewarded position (Stim 2 and Stim 4) neuron responds quite same. Lever presses activity (teal) shows, that animal anticipated the presentation of stimuli by pressing the lever, but when the presented stimuli was non rewarded rat pressed the lever with much lower frequency. Also, there are no signs that food delivery (orange) or lever pressing would affect the activity of recorded cell.

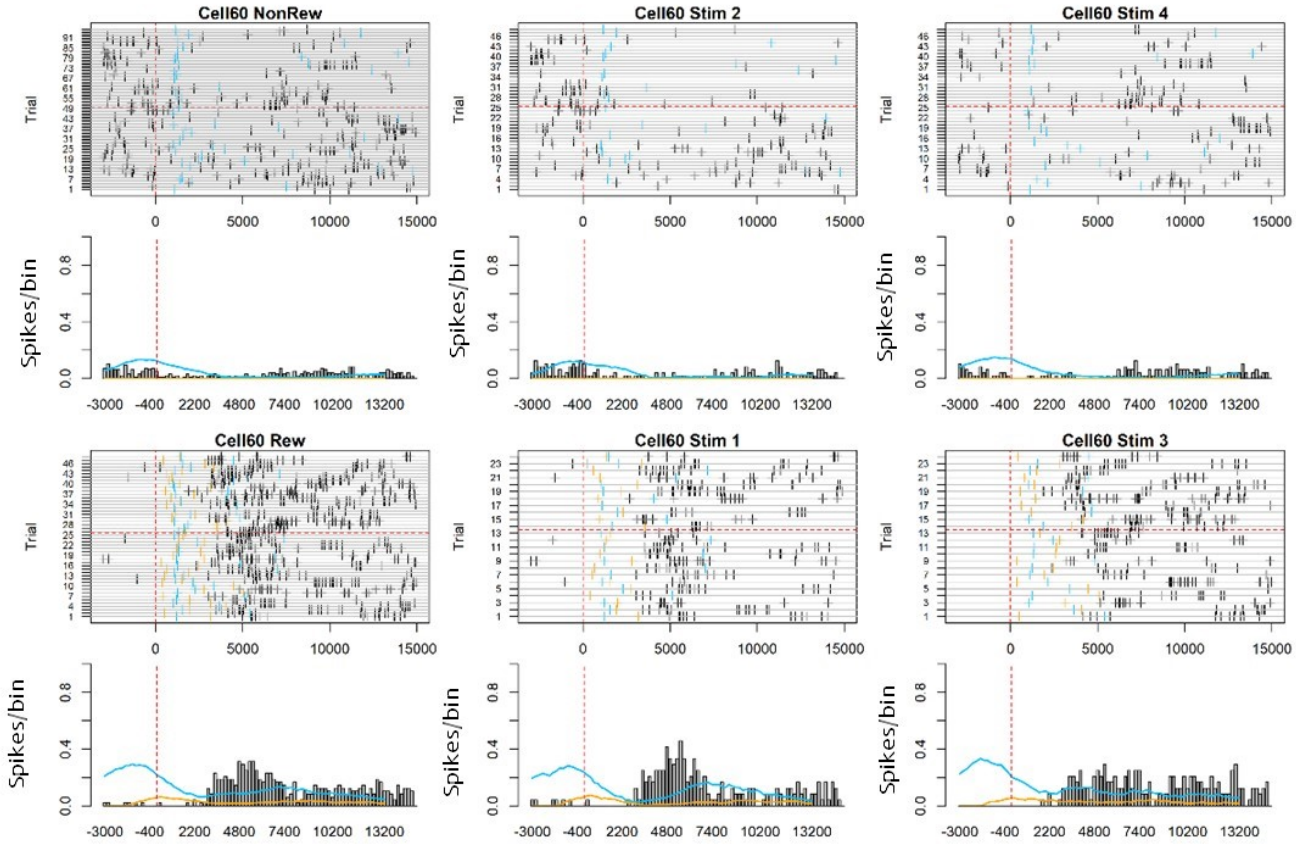
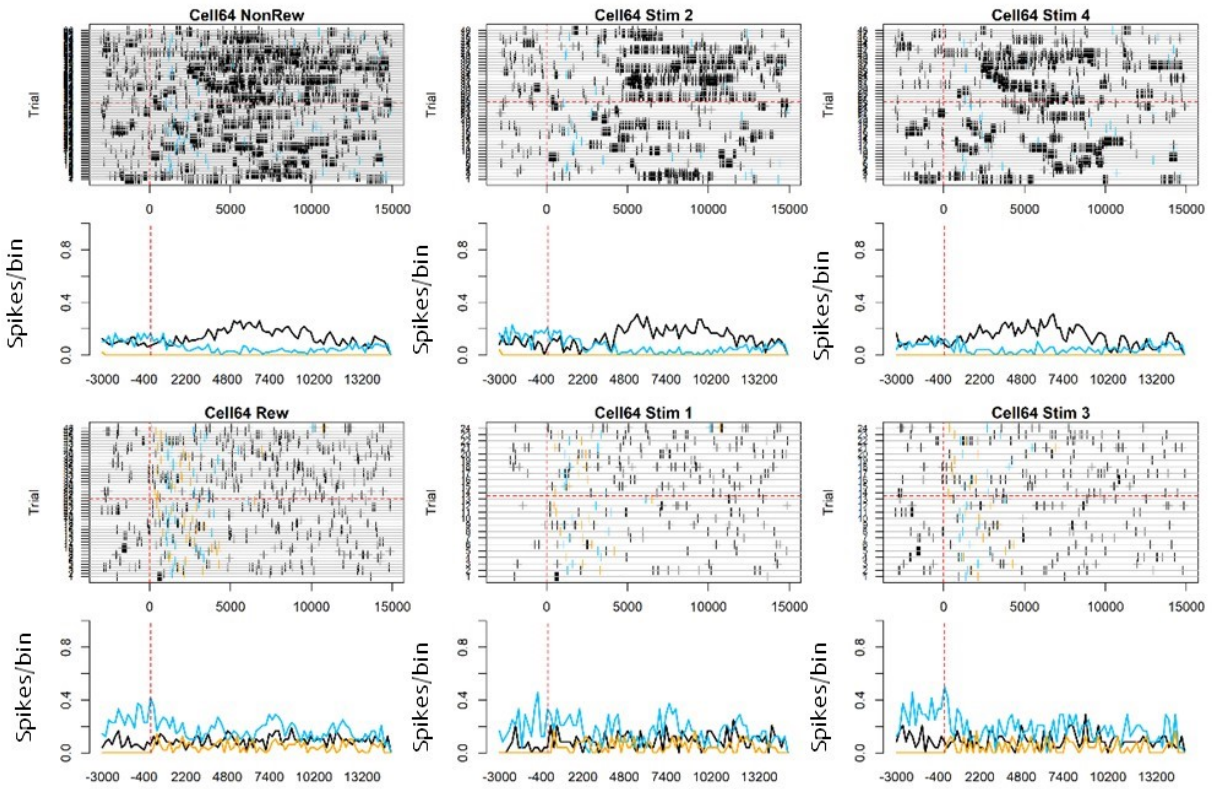


Fig. 25: Cell n.60 from rat 20. Same situation as in Fig – is represented by a cell from CA1 of the rat 20. There is also increased activity during the rewarded positions, without any apparent difference between rewarded positions. Even though the activity of neuron is little bit higher during the non-rewarded positions, than in previous case, it is still obvious that cell is responding more to rewarded positions.



Lever Feeder **Fig. 26: Cell n.64 from rat 18**. It is a representative sample of cell 64 in rat 18, that is responding to exposition of non-rewarded positions. Even though the spontaneous activity of this cell is higher than in previous cases, it fires in bursts intensively during non-rewarded positions.

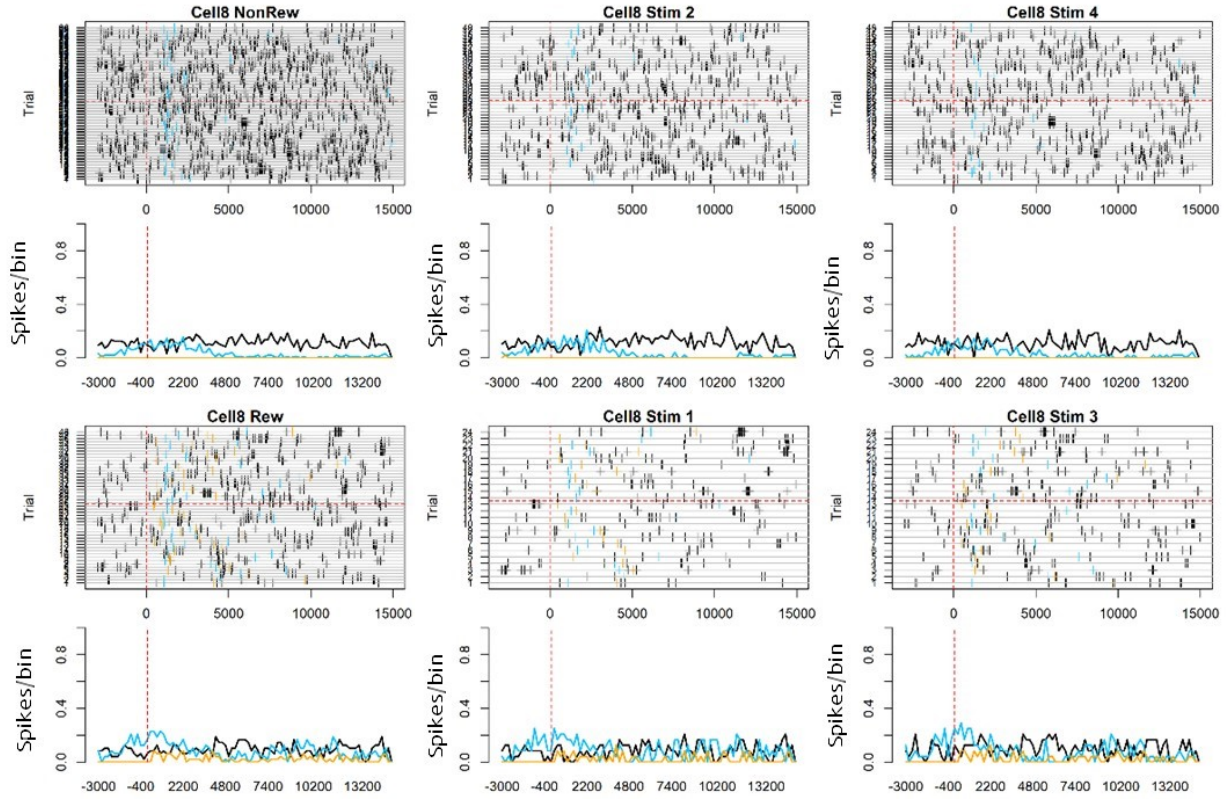


Fig. 27: Cell n.8 from rat 20. Last representative sample of neuronal activity is resembling to previous Figure, where the neuron was active during non-rewarded positions. Cell number 8 from rat 20 is also responding by same pattern to non-rewarded locations of objects.

1.26 Electrophysiological validation of tetrode location

To verify location of tetrodes in the CA1 part of hippocampus, we used a comparison of local field potentials recording with electrophysiological corelates.

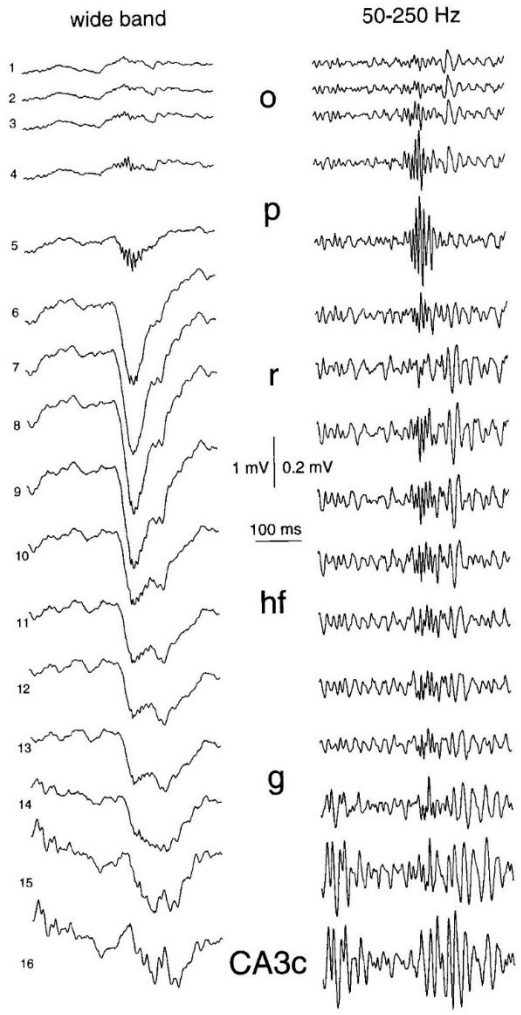


Fig 28: Electrophysiological characteristics of hippocampal layers *o - stratum oriens; p - pyramidal layer; r - radiatum layer; hf - hippocampal fissure, g- granule cell layer. Depth profile of a single sharp wave event (right) associated high-frequency oscillation (right) (A. Ylinen et al., 1995)*

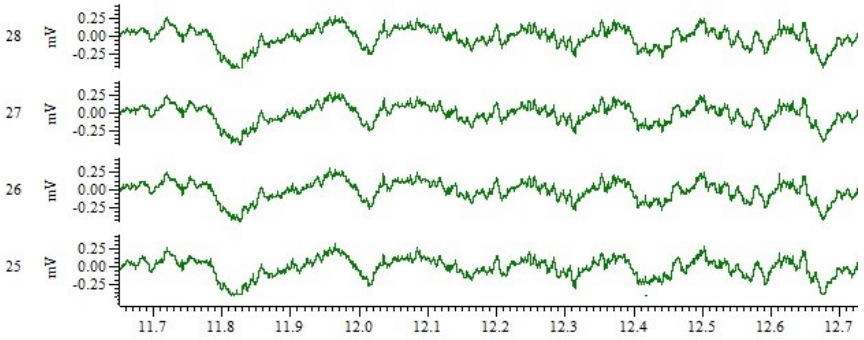


Fig. 29 Recording from final position of tetrodes.

By comparison with Fig. 28 you can see the single sharp wave event (11,8 in the recording) from the recording of final position, which is typical for Pyramidal layer (CA1) and it is almost similar as fifth sample in the compared recordings.

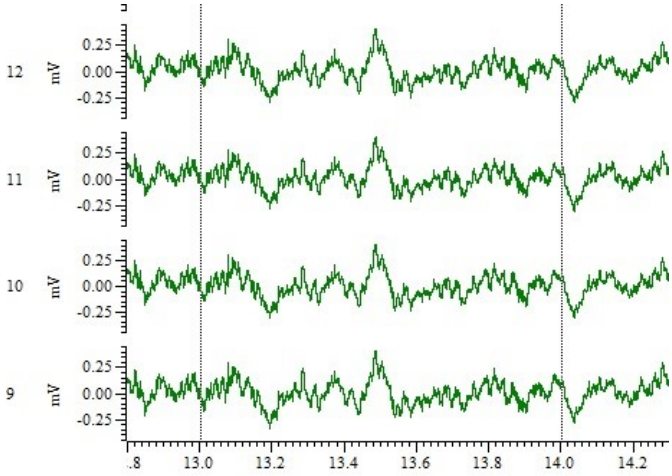


Fig.29: Theta oscillation recording from the final position of the tetrodes.

Also, other, wider, validation was the theta rhythm, which is typical for the hippocampus. As you can see, there are 7 peaks in recording during 1 second. Since hertz is the number of cycles per second, we can observe by naked eye 7 Hz frequency, which is correspond to two theta oscillation.

10. DISCUSSION

The main goal of this experiment was to clarify the function of pyramidal cells from the CA1 region of the hippocampus in the coding of positions of objects located in an inaccessible space. For this purpose, we designed a specialized behavioral task for testing inaccessible object-position discrimination. We did some modification of this previously validated task and we found out, that these modifications can facilitate or contrarily impair learning of tested rats. Two-dimensional design seemed to be more suitable for presentation of two rewarded position, as the modification of the task. Using single-unit electrophysiological methods, we shown that CA1 pyramidal cells encode the object position even in inaccessible space. These neurons responded by their increased firing activity to rewarded or non-rewarded positions, but we have not observed any specificity for single position of the stimulus. All the measured pyramidal cells exhibited some sort of categorization.

During the training of the inaccessible object position discrimination task, we used two types of stimuli. One of them was circles placed in the corners of the screen and the second one was rectangles in different distances from the center of the screen (Fig.14). We found out, that none of the rats were able to sufficiently learn to discriminate correct positions of the rectangle and to reach criterium of 60% success rate during the last twenty sessions, nor to reach a final configuration. In contrast, only one of the animals, trained for circle training was not able to reach all the criteria. Training to the rectangular object position was a modification of previously validated tasks (Klement et al., 2010) (Levcik et al., 2013). The difference was that this task used only three positions of the rectangular object (one in the center two on the edges) and only one of them was rewarded. We added a second rewarded position to see, whether the neurons will respond differently to these rewarded positions. There are a few explanations for these results. One of them is that the circles are placed in two-dimensional space in contrast to rectangles which were place in one dimension, and it may be easier to discriminate a two-dimensional change in the environment. Second is, that rectangle training has shorter distances between subjects, so animals may categorize central positions. Another modification of the task was changing the stimulus duration. In the previous version of the task, the stimulus was presented for 30 seconds. We shortened the time to 15 seconds, which was a limit for successful discrimination. We also tried a 10 second duration, but the performance of the animal could not reach a 60% of success rate five days in a row. That can be possibly explained with increased difficulty and with that connected decreased attentiveness. All of these observations gave us important information, that will help in the optimization of this task in the future experiments.

Previous pharmacological inactivation of the CA1 part of hippocampus proper shown a disruption in performance during the task used in our experiment (Levcik et al., 2013). This knowledge led us to study pyramidal cell activity of this region in the task, that would help us understand their role during the coding of the inaccessible environment. And we found that these neurons are, indeed, connected to this neuronal coding. More than 30% of neurons from measured 113 pyramidal cells were responding to the presented positions. That the activity of these cells is not connected to motor activity (such as pressing the lever) or rewarding system was shown by a comparison of this activity with animals' behavior. We found two groups of principal cells, one that was responding to rewarded positions, second that was responding to non-rewarded positions. There is no difference in firing pattern activity of neurons between two rewarded (or non-rewarded stimuli). This may point to some sort of categorization of the rewarded and non-rewarded positions.

Ho and his colleagues (Ho et al., 2008) studied whether and how pyramidal cells of CA1 respond to a surrounding moving object. They trained the rats to chaise a small car toy. The rats were rewarded in a form of transcranial stimulation when they approach the car. They found out, that in the presence of the car, place cells were remapped. This finding suggested that place cells can be modulated by moving objects located in the neighboring environment. However, they did not find specific neural activity directly encoding the object's position. Contrary to our task, this task was done in accessible space.

Teruko Danjo and her colleagues (Danjo et al., 2018) created an observational T-maze task, in which one rat (observer) made navigational decisions based on the previous choice of the other rat. Electrophysiological recording has shown that a subpopulation of pyramidal cells in CA1 of the observer encoded a particular position of the observed rat on the maze. During this phase of the experiment, the other (observed) animal was located in an inaccessible space. However, this space was accessible to the observer in the subsequent phase, when the rat navigated through the same T-maze. Thus, it was not a completely inaccessible environment. Moreover, the maze was highly familiar to the rat, since it already underwent many trials in the apparatus during previous training sessions. Another difference between this experiment and our task is that we use inanimate static visual objects while Danjo et al. used moving conspecifics to study the representation of their positions in others.

Objects positions in accessible space are also encoded by object vectors cells. Those are neurons of the entorhinal cortex, which is the main input to the CA1 region and they are active in certain distance and angle from the object. Object vector cells are the cellular basis for position mapping in the space between

objects and they provide with an allocentric vectorial representations, that animals may use to infer position and trajectories to the goals (Høydal et al., 2019).

None of the above-mentioned tasks tested the ability to discriminate positions of objects located in inaccessible space for the whole duration of the experiment. What makes our task unique is the ability to investigate the neuronal activity during object position discrimination in inaccessible space. Rats makes spatial decisions by pressing the lever in an operant box and are not allowed to explore the virtual objects and their actual locations.

11.CONCLUSIONS

In this study, we showed the role of CA1 pyramidal cells in the discrimination of positions of objects located in inaccessible space. The neurons responded to rewarded or non-rewarded positions disregarding to individual positions. This knowledge will significantly improve our understanding of coding of positions of objects located in the outside world.

We also optimized a design of the task for discrimination of two rewarded object's positions. We made some modifications of previously validated task, for the purpose to introduce two rewarded positions during object position discrimination. The two-dimensional configuration seemed to be the promising option for facilitation of the learning process during the task. This new knowledge will give us new insights to the discussed problematic, and it will be helpful in further investigations.

12.REFERENCES

Aggleton, J. P., Hunt, P. R., & Rawlins, J. N. P. (1986). The effects of hippocampal lesions upon spatial and non-spatial tests of working memory. *Behavioural Brain Research*, *19*(2), 133–146. https://doi.org/10.1016/0166-4328(86)90011-2

Alerstam, T., Hedenström, A., & Åkesson, S. (2003). Long-distance migration: Evolution and determinants. *Oikos*, *103*(2), 247–260. https://doi.org/10.1034/j.1600-0706.2003.12559.x

Alle, H., Roth, A., & Geiger, J. R. P. (2009). Energy-efficient action potentials in hippocampal mossy fibers. *Science*, *325*(5946), 1405–1408. https://doi.org/10.1126/science.1174331

Allen, K., Rawlins, J. N. P., Bannerman, D. M., & Csicsvari, J. (2012). Hippocampal place cells can encode multiple trial-dependent features through rate remapping. *Journal of Neuroscience*, *32*(42), 14752–14766. https://doi.org/10.1523/JNEUROSCI.6175-11.2012

Amaral, D., Andersen, P., O'Keefe, J., & Morris, R. (2007). *The hippocampus book*. Oxford University Press.

Anagnostaras, S. G., Gale, G. D., & Fanselow, M. S. (2001). Hippocampus and contextual fear conditioning: Recent controversies and advances. *Hippocampus*, *11*(1), 8–17. https://doi.org/10.1002/1098-1063(2001)11:1<8::AID-HIPO1015>3.0.CO;2-7

Antunes, M., & Biala, G. (2012). The novel object recognition memory: Neurobiology, test procedure, and its modifications. In *Cognitive Processing* (Vol. 13, Issue 2, pp. 93–110). Springer. https://doi.org/10.1007/s10339-011-0430-z

Backus, A. R., Schoffelen, J. M., Szebényi, S., Hanslmayr, S., & Doeller, C. F. (2016). Hippocampal-prefrontal theta oscillations support memory integration. *Current Biology*, *26*(4), 450–457. https://doi.org/10.1016/j.cub.2015.12.048

Bannister, N. J., & Larkman, A. U. (1995). Dendritic morphology of CA1 pyramidal neurones from the rat hippocampus: II. Spine distributions. *The Journal of Comparative Neurology*, *360*(1), 161–171. https://doi.org/10.1002/cne.903600112

Bartesaghi, R., & Gessi, T. (2004). Parallel activation of field CA2 and dentate gyrus by synaptically elicited perforant path volleys. *Hippocampus*, *14*(8), 948–963. https://doi.org/10.1002/hipo.20011

Bartos, M., & Elgueta, C. (2012). Functional characteristics of parvalbumin- and cholecystokinin-expressing basket cells. *The Journal of Physiology*, *590*(4), 669–681. https://doi.org/10.1113/jphysiol.2011.226175

Bast, T., Wilson, I. A., Witter, M. P., & Morris, R. G. M. (2009). From Rapid Place Learning to Behavioral Performance: A Key Role for the Intermediate Hippocampus. *PLoS Biology*, *7*(4), e1000089. https://doi.org/10.1371/journal.pbio.1000089

Battaglia, F. P., & Treves, A. (1998). Attractor neural networks storing multiple space representations: A model for hippocampal place fields. *Physical Review E - Statistical Physics, Plasmas, Fluids, and Related Interdisciplinary Topics*, *58*(6), 7738–7753. https://doi.org/10.1103/PhysRevE.58.7738

Bekkers, J. M. (2011). Pyramidal neurons. *CURBIO*, *21*, R975. https://doi.org/10.1016/j.cub.2011.10.037

Bingman, V. P. (1992). The importance of comparative studies and ecological validity for understanding hippocampal structure and cognitive function. *Hippocampus*, *2*(3), 213–219. https://doi.org/10.1002/hipo.450020302

Brandon, M. P., Koenig, J., & Leutgeb, S. (2014). Parallel and convergent processing in grid cell, head-direction cell, boundary cell, and place cell networks. *Wiley Interdisciplinary Reviews: Cognitive Science*, *5*(2), 207–219. https://doi.org/10.1002/wcs.1272

Breed, M. D., & Moore, J. (2012). Movement: Search, Navigation, Migration, and Dispersal. In *Animal Behavior* (pp. 219–252). Elsevier. https://doi.org/10.1016/b978-0-12-372581-3.00008-8

Bunsey, M., & Elchenbaum, H. (1996). Conservation of hippocampal memory function in rats and humans. *Nature*, *379*(6562), 255–257. https://doi.org/10.1038/379255a0

Burešová, O., & Bureš, J. (1981). Role of olfactory cues in the radial maze performance of rats. *Behavioural Brain Research*, *3*(3), 405–409. https://doi.org/10.1016/0166-4328(81)90009-7

Burešová, O., Bureš, J., Oitzl, M. S., & Zahálka, A. (1985). Radial maze in the water tank: An aversively motivated spatial working memory task. *Physiology and Behavior*, *34*(6), 1003–1005. https://doi.org/10.1016/0031-9384(85)90028-9

Burgess, C., Schuck, N. W., & Burgess, N. (2011). Temporal Neuronal Oscillations can Produce Spatial Phase Codes. In *Space, Time and Number in the Brain* (pp. 59–69). Elsevier Inc. https://doi.org/10.1016/B978-0-12-385948-8.00005-0

Burgess, N., Maguire, E. A., & O'Keefe, J. (2002). The human hippocampus and spatial and episodic memory. In *Neuron* (Vol. 35, Issue 4, pp. 625–641). Cell Press. https://doi.org/10.1016/S0896-6273(02)00830-9

Bussey, T. J., Holmes, A., Lyon, L., Mar, A. C., McAllister, K. A. L., Nithianantharajah, J., Oomen, C. A., & Saksida, L. M. (2012). New translational assays for preclinical modelling of cognition in schizophrenia: The touchscreen testing method for mice and rats. *Neuropharmacology*, *62*(3), 1191–1203. https://doi.org/10.1016/j.neuropharm.2011.04.011

Buzsáki, G. (2005). Theta rhythm of navigation: Link between path integration and landmark navigation, episodic and semantic memory. In *Hippocampus* (Vol. 15, Issue 7, pp. 827–840). John Wiley & Sons, Ltd. https://doi.org/10.1002/hipo.20113

Buzsáki, G., Anastassiou, C. A., & Koch, C. (2012). The origin of extracellular fields and currents-EEG, ECoG, LFP and spikes. In *Nature Reviews Neuroscience* (Vol. 13, Issue 6, pp. 407–420). Nature Publishing Group. https://doi.org/10.1038/nrn3241

Buzsáki, G., & Moser, E. I. (2013). Memory, navigation and theta rhythm in the hippocampal-entorhinal system. In *Nature Neuroscience* (Vol. 16, Issue 2, pp. 130–138). Nature Publishing Group. https://doi.org/10.1038/nn.3304

Buzsáki, G., & Wang, X.-J. (2012). Mechanisms of Gamma Oscillations. *Annual Review of Neuroscience*, *35*(1), 203–225. https://doi.org/10.1146/annurev-neuro-062111-150444

Carr, M. F., Jadhav, S. P., & Frank, L. M. (2011). Hippocampal replay in the awake state: A potential substrate for memory consolidation and retrieval. *Nature Neuroscience*, *14*(2), 147–153. https://doi.org/10.1038/nn.2732

Chozick, B. S. (1983). The behavioral effects of lesions of the hippocampus: A review. In *International Journal of Neuroscience* (Vol. 22, Issues 1–2, pp. 63–80). Informa Healthcare. https://doi.org/10.3109/00207459308987386

Cimadevilla, J. M., Wesierska, M., Fenton, A. A., & Bures, J. (2001). Inactivating one hippocampus impairs avoidance of a stable room-defined place during dissociation of arena cues from room cues by rotation of the arena. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(6), 3531–3536. https://doi.org/10.1073/pnas.051628398

Cimadevilla, Jose M., Fenton, A. A., & Bures, J. (2000). Functional inactivation of dorsal hippocampus impairs active place avoidance in rats. *Neuroscience Letters*, *285*(1), 53–56. https://doi.org/10.1016/S0304-3940(00)01019-3

Cobb, S. R., Buhl, E. H., Halasy, K., Paulsen, O., & Somogyi, P. (1995). Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature*, *378*(6552), 75–78. https://doi.org/10.1038/378075a0

Colgin, L. L., Denninger, T., Fyhn, M., Hafting, T., Bonnevie, T., Jensen, O., Moser, M. B., & Moser, E. I. (2009). Frequency of gamma oscillations routes flow of information in the hippocampus. *Nature*, *462*(7271), 353–357. https://doi.org/10.1038/nature08573

Colgin, L. L., & Moser, E. I. (2010). Gamma oscillations in the hippocampus. In *Physiology* (Vol. 25, Issue 5, pp. 319–329). American Physiological Society Bethesda, MD. https://doi.org/10.1152/physiol.00021.2010

Colombo, M., & Broadbent, N. (2000). Is the avian hippocampus a functional homologue of the mammalian hippocampus? In *Neuroscience and Biobehavioral Reviews* (Vol. 24, Issue 4, pp. 465–

484). Pergamon. https://doi.org/10.1016/S0149-7634(00)00016-6

Curley, A. A., & Lewis, D. A. (2012). Cortical basket cell dysfunction in schizophrenia. *The Journal of Physiology*, *590*(4), 715–724. https://doi.org/10.1113/jphysiol.2011.224659

D'Hooge, R., & De Deyn, P. P. (2001). Applications of the Morris water maze in the study of learning and memory. In *Brain Research Reviews* (Vol. 36, Issue 1, pp. 60–90). Elsevier. https://doi.org/10.1016/S0165-0173(01)00067-4

Danjo, T., Toyoizumi, T., & Fujisawa, S. (2018). Spatial representations of self and other in the hippocampus. *Science*, *359*(6372), 213–218. https://doi.org/10.1126/science.aao3898

Deacon, R. M. J., & Rawlins, J. N. P. (2006). T-maze alternation in the rodent. *Nature Protocols*, *1*(1), 7–12. https://doi.org/10.1038/nprot.2006.2

Descalzo, V. F., Nowak, L. G., Brumberg, J. C., McCormick, D. A., & Sanchez-Vives, M. V. (2005). Slow Adaptation in Fast-Spiking Neurons of Visual Cortex. *Journal of Neurophysiology*, *93*(2), 1111–1118. https://doi.org/10.1152/jn.00658.2004

Dragoi, G., & Tonegawa, S. (2011). Preplay of future place cell sequences by hippocampal cellular assemblies. *Nature*, *469*(7330), 397–401. https://doi.org/10.1038/nature09633

Dusek, J. A., & Eichenbaum, H. (1997). The hippocampus and memory for orderly stimulus relations. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(13), 7109–7114. https://doi.org/10.1073/pnas.94.13.7109

Ego-Stengel, V., & Wilson, M. A. (2007). Spatial selectivity and theta phase precession in CA1 interneurons. *Hippocampus*, *17*(2), 161–174. https://doi.org/10.1002/hipo.20253

Eichenbaum, H. (2017). The role of the hippocampus in navigation is memory. In *Journal of Neurophysiology* (Vol. 117, Issue 4, pp. 1785–1796). American Physiological Society. https://doi.org/10.1152/jn.00005.2017

Eichenbaum, H., Dudchenko, P., Wood, E., Shapiro, M., & Tanila, H. (1999). The hippocampus, memory, and place cells: Is it spatial memory or a memory space? In *Neuron* (Vol. 23, Issue 2, pp. 209–226). Cell Press. https://doi.org/10.1016/S0896-6273(00)80773-4

Etienne, A. S., & Jeffery, K. J. (2004). Path integration in mammals. In *Hippocampus* (Vol. 14, Issue 2, pp. 180–192). John Wiley & Sons, Ltd. https://doi.org/10.1002/hipo.10173

Fanselow, M. S., & Dong, H. W. (2010). Are the Dorsal and Ventral Hippocampus Functionally Distinct Structures? In *Neuron* (Vol. 65, Issue 1, pp. 7–19). NIH Public Access. https://doi.org/10.1016/j.neuron.2009.11.031

Fernández-Ruiz, A., Oliva, A., de Oliveira, E. F., Rocha-Almeida, F., Tingley, D., & Buzsáki, G. (2019). Long-

duration hippocampal sharp wave ripples improve memory. *Science*, *364*(6445), 1082–1086. https://doi.org/10.1126/science.aax0758

Förster, E., Zhao, S., & Frotscher, M. (2006). Laminating the hippocampus. In *Nature Reviews Neuroscience* (Vol. 7, Issue 4, pp. 259–267). https://doi.org/10.1038/nrn1882

Foster, D. J., & Wilson, M. A. (2006). Reverse replay of behavioural sequences in hippocampal place cells during the awake state. *Nature*, *440*(7084), 680–683. https://doi.org/10.1038/nature04587

Fox, S. E., & Ranck, J. B. (1981). Electrophysiological characteristics of hippocampal complex-spike cells and theta cells. *Experimental Brain Research*, *41*(3–4), 399–410. https://doi.org/10.1007/BF00238898

Freund, T. F. (2003). Interneuron Diversity series: Rhythm and mood in perisomatic inhibition. In *Trends in Neurosciences* (Vol. 26, Issue 9, pp. 489–495). Elsevier Ltd. https://doi.org/10.1016/S0166-2236(03)00227-3

Freund, T., & Kali, S. (2008). Interneurons. *Scholarpedia*, *3*(9), 4720. https://doi.org/10.4249/scholarpedia.4720

Fuhs, M. C., & Touretzky, D. S. (2006). A spin glass model of path integration in rat medial entorhinal cortex. *Journal of Neuroscience*, *26*(16), 4266–4276. https://doi.org/10.1523/JNEUROSCI.4353-05.2006

Geisler, C., Robbe, D., Zugaro, M., Sirota, A., & Buzsáki, G. (2007). Hippocampal place cell assemblies are speed-controlled oscillators. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(19), 8149–8154. https://doi.org/10.1073/pnas.0610121104

Gil, M., Ancau, M., Schlesiger, M. I., Neitz, A., Allen, K., De Marco, R. J., & Monyer, H. (2018). Impaired path integration in mice with disrupted grid cell firing. In *Nature Neuroscience* (Vol. 21, Issue 1, pp. 81–93). Nature Publishing Group. https://doi.org/10.1038/s41593-017-0039-3

Gilbert, P. E., & Kesner, R. P. (2003). Localization of Function Within the Dorsal Hippocampus: The Role of the CA3 Subregion in Paired-Associate Learning. *Behavioral Neuroscience*, *117*(6), 1385–1394. https://doi.org/10.1037/0735-7044.117.6.1385

Girardeau, G., & Zugaro, M. (2011). Hippocampal ripples and memory consolidation. In *Current Opinion in Neurobiology* (Vol. 21, Issue 3, pp. 452–459). Elsevier Current Trends. https://doi.org/10.1016/j.conb.2011.02.005

Goodridge, J. P., & Taube, J. S. (1997). Interaction between the postsubiculum and anterior thalamus in the generation of head direction cell activity. *Journal of Neuroscience*, *17*(23), 9315–9330. https://doi.org/10.1523/jneurosci.17-23-09315.1997

Gray, C. M., & McCormick, D. A. (1996). Chattering cells: Superficial pyramidal neurons contributing to the generation of synchronous oscillations in the visual cortex. *Science*, *274*(5284), 109–113. https://doi.org/10.1126/science.274.5284.109

Gulyás, A. I., Szabó, G. G., Ulbert, I., Holderith, N., Monyer, H., Erdélyi, F., Szabó, G., Freund, T. F., & Hájos, N. (2010). Parvalbumin-containing fast-spiking basket cells generate the field potential oscillations induced by cholinergic receptor activation in the hippocampus. *Journal of Neuroscience*, *30*(45), 15134–15145. https://doi.org/10.1523/JNEUROSCI.4104-10.2010

Hafting, T., Fyhn, M., Molden, S., Moser, M. B., & Moser, E. I. (2005). Microstructure of a spatial map in the entorhinal cortex. *Nature*, *436*(7052), 801–806. https://doi.org/10.1038/nature03721

Hasselmo, M. E. (2009). A model of episodic memory: Mental time travel along encoded trajectories using grid cells. *Neurobiology of Learning and Memory*, *92*(4), 559–573. https://doi.org/10.1016/j.nlm.2009.07.005

Hayashi, H., & Ishizuka, S. (1995). Chaotic responses of the hippocampal CA3 region to a mossy fiber stimulation in vitro. *Brain Research*, *686*(2), 194–206. https://doi.org/10.1016/0006-8993(95)00485-9

Henze, D. A., Borhegyi, Z., Csicsvari, J., Mamiya, A., Harris, K. D., & Buzsáki, G. (2000). Intracellular features predicted by extracellular recordings in the hippocampus in vivo. *Journal of Neurophysiology*, *84*(1), 390–400. https://doi.org/10.1152/jn.2000.84.1.390

Ho, S. A., Hori, E., Kobayashi, T., Umeno, K., Tran, A. H., Ono, T., & Nishijo, H. (2008). Hippocampal place cell activity during chasing of a moving object associated with reward in rats. *Neuroscience*, *157*(1), 254–270. https://doi.org/10.1016/j.neuroscience.2008.09.004

Høydal, Ø. A., Skytøen, E. R., Andersson, S. O., Moser, M. B., & Moser, E. I. (2019a). Object-vector coding in the medial entorhinal cortex. *Nature*, *568*(7752), 400–404. https://doi.org/10.1038/s41586-019-1077-7

Iglói, K., Zaoui, M., Berthoz, A., & Rondi-Reig, L. (2009). Sequential egocentric strategy is acquired as early as allocentric strategy: Parallel acquisition of these two navigation strategies. *Hippocampus*, *19*(12), 1199–1211. https://doi.org/10.1002/hipo.20595

Izhikevich, E. M. (2003). Simple model of spiking neurons. In *IEEE Transactions on Neural Networks* (Vol. 14, Issue 6, pp. 1569–1572). https://doi.org/10.1109/TNN.2003.820440

Jacobs, L. F. (2003). The Evolution of the Cognitive Map. *Brain, Behavior and Evolution*, *62*(2), 128–139. https://doi.org/10.1159/000072443

Jeewajee, A., Barry, C., Douchamps, V., Manson, D., Lever, C., & Burgess, N. (2014). Theta phase

precession of grid and place cell firing in open environments. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1635), 20120532. https://doi.org/10.1098/rstb.2012.0532

Jeffery, K. J., Anderson, M. I., Hayman, R., & Chakraborty, S. (2004). A proposed architecture for the neural representation of spatial context. *Neuroscience and Biobehavioral Reviews*, *28*(2), 201–218. https://doi.org/10.1016/j.neubiorev.2003.12.002

Ji, D., & Wilson, M. A. (2007). Coordinated memory replay in the visual cortex and hippocampus during sleep. *Nature Neuroscience*, *10*(1), 100–107. https://doi.org/10.1038/nn1825

Kenney, J. W., Adoff, M. D., Wilkinson, D. S., & Gould, T. J. (2011). The effects of acute, chronic, and withdrawal from chronic nicotine on novel and spatial object recognition in male C57BL/6J mice. *Psychopharmacology*, *217*(3), 353–365. https://doi.org/10.1007/s00213-011-2283-7

Kim, C. H., Romberg, C., Hvoslef-Eide, M., Oomen, C. A., Mar, A. C., Heath, C. J., Berthiaume, A. A., Bussey, T. J., & Saksida, L. M. (2015). Trial-unique, delayed nonmatching-to-location (TUNL) touchscreen testing for mice: Sensitivity to dorsal hippocampal dysfunction. *Psychopharmacology*, *232*(21–22), 3935–3945. https://doi.org/10.1007/s00213-015-4017-8

Kirk, I. J., & Mackay, J. C. (2003). The role of theta-range oscillations in synchronising and integrating activity in distributed mnemonic networks. *Cortex*, *39*(4–5), 993–1008. https://doi.org/10.1016/S0010-9452(08)70874-8

Klatzky, R. L. (1998). *Allocentric and Egocentric Spatial Representations: Definitions, Distinctions, and Interconnections* (pp. 1–17). Springer, Berlin, Heidelberg. https://doi.org/10.1007/3-540-69342-4_1

Klement, D., Levcik, D., Duskova, L., & Nekovarova, T. (2010). Spatial task for rats testing position recognition of an object displayed on a computer screen. *Behavioural Brain Research*, *207*(2), 480–489. https://doi.org/10.1016/j.bbr.2009.10.041

Knierim, J. J. (2002). Dynamic interactions between local surface cues, distal landmarks, and intrinsic circuitry in hippocampal place cells. *Journal of Neuroscience*, *22*(14), 6254–6264. https://doi.org/10.1523/jneurosci.22-14-06254.2002

Knierim, J. J., Lee, I., & Hargreaves, E. L. (2006). Hippocampal place cells: Parallel input streams, subregional processing, and implications for episodic memory. *Hippocampus*, *16*(9), 755–764. https://doi.org/10.1002/hipo.20203

Kraus, B. J., Robinson, R. J., White, J. A., Eichenbaum, H., & Hasselmo, M. E. (2013). Hippocampal "Time Cells": Time versus Path Integration. *Neuron*, *78*(6), 1090–1101. https://doi.org/10.1016/j.neuron.2013.04.015

Krupic, J., Bauza, M., Burton, S., & O'Keefe, J. (2016). Framing the grid: effect of boundaries on grid cells

and navigation. *The Journal of Physiology*, *594*(22), 6489–6499. https://doi.org/10.1113/JP270607

Kubík, Š., & Fenton, A. A. (2005). Behavioral evidence that segregation and representation are dissociable hippocampal functions. *Journal of Neuroscience*, *25*(40), 9205–9212. https://doi.org/10.1523/JNEUROSCI.1707-05.2005

Lalonde, R. (2002). The neurobiological basis of spontaneous alternation. In *Neuroscience and Biobehavioral Reviews* (Vol. 26, Issue 1, pp. 91–104). Elsevier Ltd. https://doi.org/10.1016/S0149-7634(01)00041-0

Latuske, P., Kornienko, O., Kohler, L., & Allen, K. (2018). Hippocampal Remapping and Its Entorhinal Origin. *Frontiers in Behavioral Neuroscience*, *11*, 253. https://doi.org/10.3389/fnbeh.2017.00253

Laurens, J., & Angelaki, D. E. (2018). The Brain Compass: A Perspective on How Self-Motion Updates the Head Direction Cell Attractor. In *Neuron* (Vol. 97, Issue 2, pp. 275–289). Cell Press. https://doi.org/10.1016/j.neuron.2017.12.020

Lee, I., Jerman, T. S., & Kesner, R. P. (2005). Disruption of delayed memory for a sequence of spatial locations following CA1- or CA3-lesions of the dorsal hippocampus. *Neurobiology of Learning and Memory*, *84*(2), 138–147. https://doi.org/10.1016/j.nlm.2005.06.002

Lee, I., Yoganarasimha, D., Rao, G., & Knierim, J. J. (2004). Comparison of population coherence of place cells in hippocampal subfields CA1 and CA3. *Nature*, *430*(6998), 456–459. https://doi.org/10.1038/nature02739

Lefebvre, L., Reader, S. M., & Sol, D. (2004). Brains, Innovations and Evolution in Birds and Primates. *Brain, Behavior and Evolution*, *63*(4), 233–246. https://doi.org/10.1159/000076784

Lein, E. S., Hawrylycz, M. J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., Boe, A. F., Boguski, M. S., Brockway, K. S., Byrnes, E. J., Chen, L., Chen, L., Chen, T. M., Chin, M. C., Chong, J., Crook, B. E., Czaplinska, A., Dang, C. N., Datta, S., … Jones, A. R. (2007). Genome-wide atlas of gene expression in the adult mouse brain. *Nature*, *445*(7124), 168–176. https://doi.org/10.1038/nature05453

Levcik, D., Nekovarova, T., Stuchlik, A., & Klement, D. (2013). Rats use hippocampus to recognize positions of objects located in an inaccessible space. *Hippocampus*, *23*(2), 153–161. https://doi.org/10.1002/hipo.22076

Lever, C., Burton, S., Jeewajee, A., O'Keefe, J., & Burgess, N. (2009). Boundary vector cells in the subiculum of the hippocampal formation. *Journal of Neuroscience*, *29*(31), 9771–9777. https://doi.org/10.1523/JNEUROSCI.1319-09.2009

Li, J. S., & Chao, Y. S. (2008). Electrolytic lesions of dorsal CA3 impair episodic-like memory in rats. *Neurobiology of Learning and Memory*, *89*(2), 192–198. https://doi.org/10.1016/j.nlm.2007.06.006

Liu, P., & Bilkey, D. K. (1998). Lesions of perirhinal cortex produce spatial memory deficits in the radial maze. *Hippocampus*, *8*(2), 114–121. https://doi.org/10.1002/(SICI)1098-1063(1998)8:2<114::AID-HIPO3>3.0.CO;2-L

Losonczy, A., Zemelman, B. V., Vaziri, A., & Magee, J. C. (2010). Network mechanisms of theta related neuronal activity in hippocampal CA1 pyramidal neurons. *Nature Neuroscience*, *13*(8), 967–972. https://doi.org/10.1038/nn.2597

Maccaferri, G. (2005). Stratum oriens horizontal interneurone diversity and hippocampal network dynamics. *The Journal of Physiology*, *562*(1), 73–80. https://doi.org/10.1113/jphysiol.2004.077081

Maren, S., & Quirk, G. J. (2004). Neuronal signalling of fear memory. In *Nature Reviews Neuroscience* (Vol. 5, Issue 11, pp. 844–852). Nature Publishing Group. https://doi.org/10.1038/nrn1535

Marozzi, E., & Jeffery, K. J. (2012). Place, space and memory cells. In *Current Biology* (Vol. 22, Issue 22, pp. R939–R942). Cell Press. https://doi.org/10.1016/j.cub.2012.10.022

McHugh, S. B., Campbell, T. G., Taylor, A. M., Rawlins, J. N. P., & Bannerman, D. M. (2008). A Role for Dorsal and Ventral Hippocampus in Inter-Temporal Choice Cost-Benefit Decision Making. *Behavioral Neuroscience*, *122*(1), 1–8. https://doi.org/10.1037/0735-7044.122.1.1

McNaughton, B. L., Battaglia, F. P., Jensen, O., Moser, E. I., & Moser, M. B. (2006). Path integration and the neural basis of the "cognitive map." In *Nature Reviews Neuroscience* (Vol. 7, Issue 8, pp. 663–678). Nature Publishing Group. https://doi.org/10.1038/nrn1932

Megías, M., Emri, Z., Freund, T. F., & Gulyás, A. I. (2001). Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. *Neuroscience*, *102*(3), 527–540. https://doi.org/10.1016/S0306-4522(00)00496-6

Menon, V., Boyett-Anderson, J. M., Schatzberg, A. F., & Reiss, A. L. (2002). Relating semantic and episodic memory systems. *Cognitive Brain Research*, *13*(2), 261–265. https://doi.org/10.1016/S0926-6410(01)00120-3

Milner, B. (1965). Visually-guided maze learning in man: Effects of bilateral hippocampal, bilateral frontal, and unilateral cerebral lesions. *Neuropsychologia*, *3*(4), 317–338. https://doi.org/10.1016/0028-3932(65)90005-9

Mittelstaedt, H., & Mittelstaedt, M.-L. (1982). *Homing by Path Integration* (pp. 290–297). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-68616-0_29

Mizumori, S. J. Y., Ragozzino, K. E., & Cooper, B. G. (2000). Location and head direction representation in the dorsal striatum of rats. *Psychobiology*, *28*(4), 441–462. https://doi.org/10.3758/BF03332003

Montgomery, S. M., & Buzsáki, G. (2007). Gamma oscillations dynamically couple hippocampal CA3 and

CA1 regions during memory task performance. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(36), 14495–14500. https://doi.org/10.1073/pnas.0701826104

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*, *11*(1), 47–60. https://doi.org/10.1016/0165-0270(84)90007-4

Morris, R. G.M., Garrud, P., Rawlins, J. N. P., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, *297*(5868), 681–683. https://doi.org/10.1038/297681a0

Morris, Richard G.M. (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation*, *12*(2), 239–260. https://doi.org/10.1016/0023-9690(81)90020-5

Muzzio, I. A., Kentros, C., & Kandel, E. (2009). What is remembered? Role of attention on the encoding and retrieval of hippocampal representations. *The Journal of Physiology*, *587*(12), 2837–2854. https://doi.org/10.1113/jphysiol.2009.172445

Nedelska, Z., Andel, R., Laczó, J., Vlcek, K., Horinek, D., Lisy, J., Sheardova, K., Bureš, J., & Hort, J. (2012). Spatial navigation impairment is proportional to right hippocampal volume. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(7), 2590–2594. https://doi.org/10.1073/pnas.1121588109

Nguyen, L. B., Ricciardi, T. N., & Malouf, A. T. (1996). Reinnervation of stratum lucidum by hippocampal mossy fibers is developmentally regulated. *Developmental Brain Research*, *95*(2), 184–193. https://doi.org/10.1016/0165-3806(96)00090-9

O'Keefe, J., & Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Research*, *34*(1), 171–175. https://doi.org/10.1016/0006-8993(71)90358-1

O'Keefe, John., & Nadel, L. (1978). *The hippocampus as a cognitive map*. Clarendon Press. http://arizona.openrepository.com/arizona/handle/10150/620894

Ólafsdóttir, H. F., Bush, D., & Barry, C. (2018). The Role of Hippocampal Replay in Memory and Planning. In *Current Biology* (Vol. 28, Issue 1, pp. R37–R50). Cell Press. https://doi.org/10.1016/j.cub.2017.10.073

Olton, D. S. (1987). The radial arm maze as a tool in behavioral pharmacology. *Physiology and Behavior*, *40*(6), 793–797. https://doi.org/10.1016/0031-9384(87)90286-1

Olton, D. S., Collison, C., & Werz, M. A. (1977). Spatial memory and radial arm maze performance of rats. *Learning and Motivation*, *8*(3), 289–314. https://doi.org/10.1016/0023-9690(77)90054-6

Onodera, H., Sato, G., & Kogure, K. (1986). Lesions to Schaffer collaterals prevent ischemic death of CA1 pyramidal cells. *Neuroscience Letters*, *68*(2), 169–174. https://doi.org/10.1016/0304-

3940(86)90136-9

Park, E. H., Dvorak, D., & Fenton, A. A. (2011). Ensemble place codes in hippocampus: CA1, CA3, and dentate gyrus place cells have multiple place fields in large environments. *PLoS ONE*, *6*(7), 22349. https://doi.org/10.1371/journal.pone.0022349

Poucet, B., Thinus-Blanc, C., & Muller, R. U. (1994). Place cells in the ventral hippocampus of rats. *NeuroReport*, *5*(16), 2045–2048. https://doi.org/10.1097/00001756-199410270-00014

Pyapali, G. K., Sik, A., Penttonen, M., Buzsaki, G., & Turner, D. A. (1998). Dendritic properties of hippocampal CA1 pyramidal neurons in the rat: Intracellular staining in vivo and in vitro. *Journal of Comparative Neurology*, *391*(3), 335–352. https://doi.org/10.1002/(SICI)1096-9861(19980216)391:3<335::AID-CNE4>3.0.CO;2-2

Quiroga, R. Q., Nadasdy, Z., & Ben-Shaul, Y. (2004). Unsupervised spike detection and sorting with wavelets and superparamagnetic clustering. *Neural Computation*, *16*(8), 1661–1687. https://doi.org/10.1162/089976604774201631

Redish, A. D., & Touretzky, D. S. (1998). The Role of the Hippocampus in Solving the Morris Water Maze. *Neural Computation*, *10*(1), 73–111. https://doi.org/10.1162/089976698300017908

Rodríguez, F., López, J. C., Vargas, J. P., Broglio, C., Gómez, Y., & Salas, C. (2002). Spatial memory and hippocampal pallium through vertebrate evolution: Insights from reptiles and teleost fish. *Brain Research Bulletin*, *57*(3–4), 499–503. https://doi.org/10.1016/S0361-9230(01)00682-7

Rogers, J. L., & Kesner, R. P. (2006). Lesions of the dorsal hippocampus or parietal cortex differentially affect spatial information processing. *Behavioral Neuroscience*, *120*(4), 852–860. https://doi.org/10.1037/0735-7044.120.4.852

Rubin, R. D., Watson, P. D., Duff, M. C., & Cohen, N. J. (2014). The role of the hippocampus in flexible cognition and social behavior. In *Frontiers in Human Neuroscience* (Vol. 8, Issue SEP, p. 742). Frontiers Media S. A. https://doi.org/10.3389/fnhum.2014.00742

Sargolini, F., Fyhn, M., Hafting, T., McNaughton, B. L., Witter, M. P., Moser, M. B., & Moser, E. I. (2006). Conjunctive representation of position, direction, and velocity in entorhinal cortex. *Science*, *312*(5774), 758–762. https://doi.org/10.1126/science.1125572

Schultz, C., & Engelhardt, M. (2014). Anatomy of the Hippocampal Formation. In *The Hippocampus in Clinical Neuroscience* (Vol. 34, pp. 6–17). S. Karger AG. https://doi.org/10.1159/000360925

SCOVILLE, W. B., & MILNER, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery, and Psychiatry*, *20*(1), 11–21. http://www.ncbi.nlm.nih.gov/pubmed/13406589

Sirvio, J., Larson, J., Quach, C. N., Rogers, G. A., & Lynch, G. (1996). Effects of pharmacologically facilitating glutamatergic transmission in the trisynaptic intrahippocampal CIRCUIT. *Neuroscience*, *74*(4), 1025–1035. https://doi.org/10.1016/0306-4522(96)00170-4

Skaggs, W. E., McNaughton, B. L., Wilson, M. A., & Barnes, C. A. (1996). Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences. *Hippocampus*, *6*(2), 149–172. https://doi.org/10.1002/(SICI)1098-1063(1996)6:2<149::AID-HIPO6>3.0.CO;2-K

Solstad, T., Moser, E. I., & Einevoll, G. T. (2006). From grid cells to place cells: A mathematical model. *Hippocampus*, *16*(12), 1026–1031. https://doi.org/10.1002/hipo.20244

Southan, A. P., & Robertson, B. (1998). Modulation of inhibitory post-synaptic currents (IPSCs) in mouse cerebellar Purkinje and basket cells by snake and scorpion toxin K+ channel blockers. *British Journal of Pharmacology*, *125*(6), 1375–1381. https://doi.org/10.1038/sj.bjp.0702218

Spence, K. W. (1950). Cognitive versus stimulus-response theories of learning. *Psychological Review*, *57*(3), 159–172. https://doi.org/10.1037/h0058250

Stackman, R. W., & Taube, J. S. (1998). Firing properties of rat lateral mammillary single units: Head direction, head pitch, and angular head velocity. *Journal of Neuroscience*, *18*(21), 9020–9037. https://doi.org/10.1523/JNEUROSCI.18-21-09020.1998

Staff, N. P., & Spruston, N. (2003). Intracellular correlate of EPSP-spike potentiation in CA1 pyramidal neurons is controlled by GABAergic modulation. *Hippocampus*, *13*(7), 801–805. https://doi.org/10.1002/hipo.10129

Stepan, J., Dine, J., & Eder, M. (2015). Functional optical probing of the hippocampal trisynaptic circuit in vitro: Network dynamics, filter properties, and polysynaptic induction of CA1 LTP. In *Frontiers in Neuroscience* (Vol. 9, Issue APR, p. 160). Frontiers Research Foundation. https://doi.org/10.3389/fnins.2015.00160

Stuchlik, A., & Bures, J. (2002). Relative contribution of allothetic and idiothetic navigation to place avoidance on stable and rotating arenas in darkness. *Behavioural Brain Research*, *128*(2), 179–188. https://doi.org/10.1016/S0166-4328(01)00314-X

Svoboda, J., Telensky, P., Blahna, K., Vodicka, M., & Stuchlik, A. (2015). The role of rat posterior parietal cortex in coordinating spatial representations during place avoidance in dissociated reference frames on a continuously rotating arena (Carousel). *Behavioural Brain Research*, *292*, 1–9. https://doi.org/10.1016/j.bbr.2015.05.008

Talpos, J. C., McTighe, S. M., Dias, R., Saksida, L. M., & Bussey, T. J. (2010). Trial-unique, delayed nonmatching-to-location (TUNL): A novel, highly hippocampus-dependent automated touchscreen

test of location memory and pattern separation. *Neurobiology of Learning and Memory*, *94*(3), 341–352. https://doi.org/10.1016/j.nlm.2010.07.006

Talpos, J. C., Winters, B. D., Dias, R., Saksida, L. M., & Bussey, T. J. (2009). A novel touchscreen-automated paired-associate learning (PAL) task sensitive to pharmacological manipulation of the hippocampus: A translational rodent model of cognitive impairments in neurodegenerative disease. *Psychopharmacology*, *205*(1), 157–168. https://doi.org/10.1007/s00213-009-1526-3

Taube, J. S. (2007). The Head Direction Signal: Origins and Sensory-Motor Integration. *Annual Review of Neuroscience*, *30*(1), 181–207. https://doi.org/10.1146/annurev.neuro.29.051605.112854

Thomas, M. J., Watabe, A. M., Moody, T. D., Makhinson, M., & O'Dell, T. J. (1998). Postsynaptic complex spike bursting enables the induction of LTP by theta frequency synaptic stimulation. *Journal of Neuroscience*, *18*(18), 7118–7126. https://doi.org/10.1523/jneurosci.18-18-07118.1998

Tolman, E. C. (1948). Cognitive maps in rats and men. *Psychological Review*, *55*(4), 189–208. https://doi.org/10.1037/h0061626

Tulving, E., & Markowitsch, H. J. (1998). Episodic and declarative memory: Role of the hippocampus. *Hippocampus*, *8*(3), 198–204. https://doi.org/10.1002/(SICI)1098-1063(1998)8:3<198::AID-HIPO2>3.0.CO;2-G

van Goethem, N. P., van Hagen, B. T. J., & Prickaerts, J. (2018). Assessing spatial pattern separation in rodents using the object pattern separation task. *Nature Protocols*, *13*(8), 1763–1792. https://doi.org/10.1038/s41596-018-0013-x

Vorhees, C. V., & Williams, M. T. (2006). Morris water maze: Procedures for assessing spatial and related forms of learning and memory. *Nature Protocols*, *1*(2), 848–858. https://doi.org/10.1038/nprot.2006.116

Walther, C. (2002). Hippocampal terminology: Concepts, misconceptions, origins. In *Endeavour* (Vol. 26, Issue 2, pp. 41–44). Elsevier Ltd. https://doi.org/10.1016/S0160-9327(02)01412-6

Wang, C., Chen, X., & Knierim, J. J. (2020). Egocentric and allocentric representations of space in the rodent brain. In *Current Opinion in Neurobiology* (Vol. 60, pp. 12–20). Elsevier Ltd. https://doi.org/10.1016/j.conb.2019.11.005

Whishaw, I. Q., & Mittleman, G. (1986). Visits to starts, routes, and places by rats (Rattus norvegicus) in swimming pool navigation tasks. *Journal of Comparative Psychology (Washington, D.C. : 1983)*, *100*(4), 422–431. https://doi.org/10.1037/0735-7036.100.4.422

Wijekoon, J. H. B., & Dudek, P. (2007). Spiking and bursting firing patterns of a compact VLSI cortical neuron circuit. *IEEE International Conference on Neural Networks - Conference Proceedings*, 1332–

1337. https://doi.org/10.1109/IJCNN.2007.4371151

Witter, M. (2012). Hippocampus. In *The Mouse Nervous System* (pp. 112–139). Elsevier Inc. https://doi.org/10.1016/B978-0-12-369497-3.10005-6

Witter, M. P., & Amaral, D. G. (2004). *Hippocampal Formation*. 635–704. https://doi.org/10.1016/B978-012547638-6/50022-5

Wood, E. R., Dudchenko, P. A., Robitsek, R. J., & Eichenbaum, H. (2000). Hippocampal neurons encode information about different types of memory episodes occurring in the same location. *Neuron*, *27*(3), 623–633. https://doi.org/10.1016/S0896-6273(00)00071-4

Yamaguchi, Y., Aota, Y., McNaughton, B. L., & Lipa, P. (2002). Bimodality of Theta Phase Precession in Hippocampal Place Cells in Freely Running Rats. *Journal of Neurophysiology*, *87*(6), 2629–2642. https://doi.org/10.1152/jn.2002.87.6.2629

Ylinen, A., Bragin, A., Nadasdy, Z., Jando, G., Szabo, I., Sik, A., & Buzsaki, G. (1995). Sharp wave-associated high-frequency oscillation (200 hz) in the intact hippocampus: Network and intracellular mechanisms. *Journal of Neuroscience*, *15*(1 I), 30–46. https://doi.org/10.1523/jneurosci.15-01-00030.1995

Ylinen, Aarne, Soltész, I., Bragin, A., Penttonen, M., Sik, A., & Buzsáki, G. (1995). Intracellular correlates of hippocampal theta rhythm in identified pyramidal cells, granule cells, and basket cells. *Hippocampus*, *5*(1), 78–90. https://doi.org/10.1002/hipo.450050110

Zola-Morgan, S., & Squire, L. R. (1986). Memory Impairment in Monkeys Following Lesions Limited to the Hippocampus. *Behavioral Neuroscience*, *100*(2), 155–160. https://doi.org/10.1037/0735-7044.100.2.155

1.27 Figures

Fig.1: **Difference between egocentric and allocentric navigation**. Picture by author

Fig2**: Schematic picture of the connection circuit of the hippocampal formation***. Inspired by*

Amaral, D., Andersen, P., O'Keefe, J., & Morris, R. (2007). *The hippocampus book*. Oxford University Press.

Fig 3: **Schematic picture of the rat brain shows the localization and size of the hippocampus***. Picture by author.*

Fig. 4: **Scheme of a trisynaptic circuit.** Picture by author.

Fig 5: **Schematic overview of the memory types.** Picture by author inspired by Tulving, E., & Markowitsch, H. J. (1998). Episodic and declarative memory: Role of the hippocampus. *Hippocampus*, *8*(3), 198–204. https://doi.org/10.1002/(SICI)1098-1063(1998)8:3<198::AID-HIPO2>3.0.CO;2-G

Fig. 6a: **Scheme of T-maze and radial maze**. Picture by author

Fig. 6b**: Schematic picture of The Morris water maze and Carousel maze.** Picture by author

Fig 7**. Object-in-place paired-associative learning task.** Taken from Talpos, J. C., Winters, B. D., Dias, R., Saksida, L. M., & Bussey, T. J. (2009). A novel touchscreen-automated paired-associate learning (PAL) task sensitive to pharmacological manipulation of the hippocampus: A translational rodent model of cognitive impairments in neurodegenerative disease. *Psychopharmacology*, *205*(1), 157–168. https://doi.org/10.1007/s00213-009-1526-3

Fig. 8**: Trial-unique nonmatching-to-location task apparatus.** Taken from Talpos, J. C., McTighe, S. M., Dias, R., Saksida, L. M., & Bussey, T. J. (2010). Trial-unique, delayed nonmatching-to-location (TUNL): A novel, highly hippocampus-dependent automated touchscreen test of location memory and pattern separation. *Neurobiology of Learning and Memory*, *94*(3), 341–352. https://doi.org/10.1016/j.nlm.2010.07.006

Fig. 9**: Schema of the apparatus for inaccessible object position discrimination.** Picture by author.

Fig. 10**: Place field scheme.** Picture by author.

Fig. 11: **Spatial related activity differences between Grid cells, Head direction cell, Place cell and Boundary vector cell during Open field task**. Taken from Brandon, M. P., Koenig, J., & Leutgeb, S. (2014). Parallel and convergent processing in grid cell, head-direction cell, boundary cell, and place cell networks. *Wiley Interdisciplinary Reviews: Cognitive Science*, *5*(2), 207–219. https://doi.org/10.1002/wcs.1272

Fig 12: **Schematic picture of theta precession.** From Marozzi, E., & Jeffery, K. J. (2012). Place, space and memory cells. In *Current Biology* (Vol. 22, Issue 22, pp. R939–R942). Cell Press. https://doi.org/10.1016/j.cub.2012.10.022

Fig. 13. **Difference between complex-spike cells and theta cells.** Taken from Amaral, D., Andersen, P., O'Keefe, J., & Morris, R. (2007). *The hippocampus book*. Oxford University Press.

Fig.14a **Scheme of the size of an operant chamber***. Taken* from Nekovárová, T., & Klement, D. (2006). Operant behavior of the rat can be controlled by the configuration of objects in an animated scene displayed on a computer screen. *Physiological research*, *55*(1), 105.

Fig. 14b **Photo of the apparatus during the learning session** . Photo by Kristýna Malenínská.

Fig.15: **Types of stimuli.** Picture by author

Fig. 16**: Localization of the initial position of the tetrodes marked with a red dot.** Adapted from Paxinos, G., & Watson, C. (2007). *The rat brain in stereotaxic coordinates in stereotaxic coordinates*.nElsevier.

Fig.17: **Versa Drive from Neuralynx***. From* 8 Tetrode VersaDrive Revision 1.0 August 2, 2018; Neuralynx, Inc. 105 Commercial Drive*, www.Neuralynx.com*

Fig. 18**: Scheme of recording** By Maren, S., & Quirk, G. J. (2004). Neuronal signalling of fear memory. In *Nature Reviews Neuroscience* (Vol. 5, Issue 11, pp. 844–852). Nature Publishing Group. https://doi.org/10.1038/nrn1535

Fig. 19: ***Scheme of data analysis from*** Quiroga, R. Q., Nadasdy, Z., & Ben-Shaul, Y. (2004). Unsupervised spike detection and sorting with wavelets and superparamagnetic clustering. *Neural Computation*, *16*(8), 1661–1687. https://doi.org/10.1162/089976604774201631

Fig. 20: ***Learning phase graph*****.** By author.

Fig. 21 **Graph of percentage of lever presses during a recording.** *By author.*

Fig.22**: Percentual representation of recorded cells.** *By author.*

Fig.22b**: Percentual representation of firing activity of pyramidal cells***. By author*

Fig. 23: **Complex spiking pyramidal cell.** *By author.*

Fig.24 **Cell n. 26 from rat 18. *??????***

Fig. 25**: Cell n.60 from rat 20.**

Fig. 26: **Cell n.64 from rat 18**.

Fig. 27**: Cell n.8 from rat 20.**

Fig 28: **Electrophysiological characteristics of hippocampal layers.** From Ylinen, Aarne, Soltész, I., Bragin, A., Penttonen, M., Sik, A., & Buzsáki, G. (1995). Intracellular correlates of hippocampal theta rhythm in identified pyramidal cells, granule cells, and basket cells. *Hippocampus*, *5*(1), 78–90. https://doi.org/10.1002/hipo.450050110

Fig. 29 **Recording from final position of tetrodes.** By Author

Fig.29: **Theta oscillation recording from final position of the tetrodes.** By author